

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets

(11) Publication number:

0 164 273**A2**

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 85304059.0

(22) Date of filing: 07.06.85

(51) Int. Cl.: **C 12 N 15/00, C 12 P 21/02,**
C 12 N 1/20, C 07 K 7/10,
A 61 K 37/24
 //(C12N1/20, C12R1:19)

(30) Priority: 08.06.84 JP 116605/84
 08.06.84 JP 116606/84

(43) Date of publication of application: 11.12.85
 Bulletin 85/50

(84) Designated Contracting States: AT BE CH DE FR GB IT
 LI LU NL SE

(71) Applicant: Suntory Limited, 1-40 Dojimahama 2-chome
 Kita-ku, Osaka-shi Osaka-fu 530 (JP)
 Applicant: Matsuo, Hisayuki, 6653 Ooaza-Kihara
 Kiyotake-cho, Miyazaki-gun Miyazaki-ken (JP)

(72) Inventor: Matsuo, Hisayuki, 6653, Ooaza Kihara
 Kiyotake-cho, Miyazaki-gun Miyazaki-ken (JP)
 Inventor: Kangawa, Kenji, 1520-24 Kanoukou
 Kiyotake-cho, Miyazaki-gun Miyazaki-ken (JP)
 Inventor: Hayashi, Yujiro, 31-2 Ikeda 1-chome,
 Neyagawa-shi Osaka (JP)
 Inventor: Oikawa, Shinzo,
 1-8-703 Ooharano-higashisakaidani 1-chome,
 Nishikyo-ku Kyoto-shi Kyoto (JP)
 Inventor: Oshima, Takehiro, 17-10-383 Besshohonmachi,
 Takatsuki-shi Osaka (JP)
 Inventor: Tanaka, Shoji,
 8-46 Minamikaneden-cho 1-chome, Suita-shi Osaka (JP)
 Inventor: Nakazato, Hiroshi, Misawa-hai-ten E-505 13,
 Misawa-cho, Ibaraki-shi Osaka (JP)
 Inventor: Tawaragi, Yasunori,
 Rabiannu-REPO 203 1-10 Shimohozumi 2-chome,
 Ibaraki-shi Osaka (JP)

(74) Representative: Ford, Michael Frederick et al,
 MEWBURN ELLIS & CO. 2/3 Cursitor Street, London
 EC4A 1BQ (GB)

(54) New peptide and gene coding for same.

(57) Disclosed is a DNA fragment comprising a base sequence coding for a peptide occurring in human atrium cordis and having diuretic action or a precursor of the peptide, plasmids containing the DNA fragment, micro-organisms transformed with the plasmid, and a process for production of the peptide using the transformant.

Also disclosed is a new peptide consisting of 126 amino acids occurring in human atrium cordis and a precursor thereof. The peptide has notable diuretic action and hypotensive or antihypertensive actions.

10
 Asn Pro Met Tyr Asn Ala Val Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp
 20
 30
 His Leu Glu Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser Glu
 40
 50
 Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val Pro Pro Trp Thr Gly
 60
 70
 Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser
 80
 90
 Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu
 100
 110
 Arg Arg Ser Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly
 120
 126
 Cys Asn Ser Phe Arg Tyr

EP 0 164 273 A2

NEW PEPTIDE AND GENE CODING FOR SAME

The present invention relates to a novel peptide, a gene coding for the peptide, a process for production of the peptide using the gene inserted to an expression vector, and use of the peptide.

5

A normal regulation of the blood pressure in a human body is important for the maintenance of personal health, and various physical and humoral factors contribute to this regulation of the blood vessels, etc. The humoral factors include, for example, the reni-
10 ngiotensin-aldosterone system, catecholamines, prostaglandins, kinin-kallikrein system, and natriuretic hormones including ouabain-like substances. Herein the term "natriuretic" will denote selective excretion of
15 sodium cation relating to potassium cation.

Granules morphologically similar to granules present in peptide hormone-producing cells are found in human atrium (J.D. Jamieson and G.E. Palade, J. Cell Biol., 23, 151, 1964). An extract of rat atrium
20 and granules contained therein are known to show natriuretic action in rats (A.J. DeBold et. al., Life Science, 28, 89, 1981; R. Keeller, Can. J. Physiol. Pharmacol., 60, 1078, 1982). Recently Mark G., Currie S. et. al. suggested peptide-like substances with a
25 molecular weight of 20,000 to 30,000, or not more than 10,000, present in the atrium of humans, rabbits, swine, and rats, and having natriuretic action (Science, 221, 71-73, 1983).

Moreover, a peptide consisting of 28 amino
30 acid derived from rat atrium cordis was identified (Biochem. Biophys. Res. Commun.; vol 117, No. 3, P 859-865, 1983). The present inventors found a new peptide consisting of 28 amino acids from human atrium

cordis, referred to as "α-human atrial natriuretic polypeptide" and abbreviated as "α-hANP" (Biochem. Biophys. Res. Commun. Vol 118, No. 1, P 131-139, 1984, U.S.P. Serial No. 685151 of the present inventors.

5 The α-hANP has following amino acid sequence:

1	10
H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Met-	
20	
Asp-Arg-Ile-Gly-Ala-Glu-Ser-Gly-Leu-Gly-Cys-Asn-	
28	
Ser-Phe-Arg-Tyr-OH	

wherein Cys at the 7 position and Cys at the 23 position are bonded through a disulfide bond.

Various kinds of physiologically active peptides are known to be derived in vivo from their
 15 precursors by the action of an endopeptidase or exopeptidase, rather than produced directly. For example, β-endorphin and γ-lipotropin are derived from their precursor β-lipotropin, and β-melanocyte-stimulating hormone is derived from its precursor γ-lipotropin
 20 (Takahashi H. et. al., FEBS Lett. 135, 97-102, 1981).

On the basis of the above-mentioned findings, the present inventors expected that the above-mentioned α-hANP would be derived from its precursor, and actually
 25 found a new peptide derived from human atrium cordis consisting of 126 amino acids including 28 amino acids of the α-hANP. Moreover, the present inventors successfully obtained a gene coding for the new peptide, and constructed plasmid containing the gene which expresses
 30 in a microorganism.

Therefore, the present invention provides a new peptide consisting of 126 amino acids and having diuretic action and hypotensive or antihypertensive action. The peptide is hereinafter referred to as "γ-human atrial
 35 natriuretic polypeptide" and abbreviated as γ-hANP".

There is also provided a DNA fragment comprising a base sequence coding for a peptide occurring in human

atrium cordis and having diuretic action or a precursor of the peptide.

There is also provided plasmids containing a promotor region, SD sequence, and the above-mentioned DNA fragment, and capable of producing a peptide coded by the DNA fragment in a microorganism.

There is also provided a microorganism transformed with the plasmid.

The present invention further provides a process for the production of the γ -hANP or precursor peptide thereof, by culturing the transformed microorganism in a culture medium and recovering the γ -hANP or precursor from the cultured cells or medium, or converting either such peptide to or from an acid addition salt.

The present invention further extends to provide a pharmaceutical composition containing the γ -hANP as a diuretic or hypotensor and extends to medical use of γ -hANP.

The invention will be further explained and embodiments and examples set forth, in the description below, referring to the drawings in which:

Figure 1 represents an amino acid sequence of γ -hANP consisting of 126 amino acids;

Figure 2 represents an amino acid sequence of a precursor of the γ -hANP, which sequence consists of 151 amino acids wherein a sequence of from 26 to 151 corresponds to the sequence of the γ -hANP set forth in Figure 1;

Figure 3 represents a base sequence coding for the amino acid sequence set forth in Figure 1;

Figure 4 represents a base sequence coding for the amino acid sequence set forth in Figure 2;

Figure 5 represents a sequence of a cDNA fragment cloned into plasmid pANP82 including a 5' non-coding region, a coding region corresponding to the base sequence set forth in Figure 4, and a 3' non-coding region;

Figure 6 represents a construction process of

plasmid pS220 from a plasmid pS20 and a DNA fragment containing a base sequence coding for amino acid sequence of γ -hANP;

5 Figure 7 represents a result of electrophoresis showing expression of a γ -hANP gene.

Figure 8 represents a construction process of plasmid pS223-3 from plasmid pS83-3 and a DNA fragment containing a base sequence coding for amino acid sequence of γ -hANP, and a construction process of plasmid pS224-3
10 from plasmid pS84-3 and the same DNA fragment;

Figure 9 represents base sequences of probes used for selection of DNA fragments coding for amino acid sequence of γ -hANP;

Figure 10 represents a strategy used for sequencing
15 DNAs of plasmid phANP1 and phANP82;

Figure 11 represents graphs showing diuretic action of γ -hANP;

Figure 12 represents a chart showing the hypotensive action of the γ -hANP;

20 Figure 13 represents a chromatogram showing an elution profile using Sephadex G-75 during the purification of the γ -hANP;

Figure 14 represents an elution profile of a high performance liquid chromatography (HPLC) during the
25 purification of the γ -hANP; and

Figure 15 represents an elution profile of HPLC of purified γ -hANP.

1. Identification of γ -hANP Gene

30 RNA were extracted from human atrium cordis by a method disclosed in Chirgwin (Chirgwin, J. M. et. al., Biochemistry 18, 5294-5299, 1979), and enriched for poly (A)⁺ RNA (mRNA) with an oligo (dT) cellulose column. The poly (A)⁺ RNA were used to prepare a cDNA
35 library according to the Okayama-Berg method (Mol. Cell. Biol. 2, 161-170, 1982). The library was screened with a mixture of probes consisting of synthesized 14 mer-

oligonucleotides labeled with ^{32}P coding for an amino acid sequence of from 12 to 14 of α -hANP (Fig. 9), and 23 clones were selected which hybridized with the probes. Among the 23 clones, 8 clones were used to
5 extract plasmids which were then sequenced with the mixture of probes by the dideoxy method. As a result, all of the plasmids tested contained a base sequence coding for α -hANP. Among these plasmids, two plasmids containing a longer insert DNA fragment, i.e., plasmids
10 phANP1 and phANP82, having about 980 base pairs (pb) and about 850 pb, respectively, were selected, and the insert fragments were completely sequenced by the Maxam and Gilbert method (Meth. Enzym., 65, 499-560, 1980) and the method disclosed by Sanger et. al. (Proc. Natl.
15 Acad. Sci. USA, 74, 5463-5467, 1977). Both fragments had a long open reading frame which starts from a translation initiation codon ATG and ends at a translation stop codon TGA. However, the fragment in the plasmid phANP82 was shorter by about 100 bp than the
20 fragment in the plasmid phANP1. A base sequence of the fragment in the plasmid phANP82 is shown in Fig. 5. Figure 5 also contains an amino acid sequence expected from the base sequence of the open reading frame. The expected amino acid sequence consists of 151 amino acids
25 and includes an amino acid sequence of α -hANP already established, which sequence is shown in a box in Fig. 5.

On the other hand, a peptide having a molecular weight of about 13,000 Dalton was isolated from human atrium cordis. The peptide was sequenced, and determined
30 to have an amino acid sequence set forth in Fig. 1 (described below in detail).

The amino acid sequence of the peptide extracted from human atrium cordis is identical with an expected amino acid sequence from the 26 position to 151 position
35 in Fig. 5. This means that the cDNA fragment in the plasmid phANP82 contains a base sequence coding for γ -hANP. the cDNA fragment also contains an additional

base sequence coding for an amino acid sequence from Met at position 1 to Ala at position 25 in Fig. 5. The amino acid sequence from 1 to 25 is believed to be a signal peptide which participates in the secretion of a peptide. As shown above and in Fig. 5, the cDNA region in the plasmid phANP82 consists of a 5' non-translation region of 90 bp, a coding region of 453 bp, and a 3' non-translation region of 300 bp. The coding region consists of a region coding for a signal sequence of from 1 to 25 in amino acid position and a region coding for γ -hANP peptide of from 26 to 151 in amino acid position. The region coding for γ -hANP peptide includes a region coding for α -hANP peptide of from 124 to 151 in amino acid position.

The above-mentioned relationship between α -hANP and γ -hANP supports the expectation that γ -hANP is a precursor of α -hANP. When α -hANP is derived from γ -hANP, an amide bond of the Pro-Arg sequence present at 122 and 123 in Fig. 5, i.e., an amide bond between Arg-Ser at 123 and 124, has to be cleaved. This possibility is strongly suggested from a phenomenon wherein gastrin releasing peptide is derived from its precursor by cleavage of an amide bond of the Pro-Arg sequence present in the precursor (Reave, J.R. et. al., J. Biol. Chem. 258, 5582-5588, 1983; and Minamino, N. et. al., Biochem. Biophys. Res. Commun. 119, 14-20, 1984). From the similarity between γ -hANP and gastrin releasing peptide in the presence of the Pro-Arg sequence, α -hANP is reasonably considered to be formed in vivo from γ -hANP by processing (perhaps by enzymic action).

2. Construction of Recombinant DNA

As DNA fragments cloned into plasmide phANP1 and phANP82 contain a base sequence coding for γ -hANP, and therefore α -hANP, both of which have natriuretic or diuretic action and hypotensive or antihypertensive action, the DNA fragments are useful for the industrial production of γ -hANP and α -hANP and other diuretic

peptides having an amino acid sequence present in the γ -hANP.

For the construction of a recombinant DNA containing a base sequence coding for γ -hANP and useful for expression of the γ -hANP gene, a restriction enzyme EcoRI cleavage site and a translation initiation codon ATG were incorporated into an upstream site adjacent to the γ -hANP gene in the DNA fragment by in vitro mutation (Zoller, M. J. & Smith, M., Nucl. Acid. Res. 10, 6487, 1982). RF-DNA of M13mp8 and DNA of plasmid pANP1 were cleaved with Pst I to obtain cleaved RF-DNA of M13mp8 and a DNA fragment of about 700 bp containing the γ -hANP gene, respectively. Both DNA fragments were ligated with T4 DNA ligase, and transformed into E. coli JM103. The transformed E. coli was cultured to obtain a single strand phage containing the 700 bp fragment. The single strand phage DNA was used to form a hetero duplex with a chemically synthesized 36 mer single strand DNA. The synthesized DNA consists of a base sequence GAATTCATG coding for the above-mentioned EcoRI site and the translation initiation codon ATG and corresponding to a sequence ACC AGA GCT in Fig. 5 (shown by a dotted line), a base sequence of 12 bases identical with a sequence upstream of the dot-lined sequence, and a base sequence of 15 bases identical with a sequence downstream of the dot-lined sequence. The single strand DNA partially having the hetero duplex was converted to a double strand DNA according to a conventional method with a DNA polymerase I Klenom fragment (Zoller, J.M. & Smith, M., Nucl. Acid Res. 10, 6487, 1982). The formed double strand DNA was transformed into E. coli JM103. To select plasmid DNA containing a base sequence GAATTCATG in place of the original base sequence ACCAGAGCT, screening was carried out by culturing the transformed E. coli, obtaining DNAs, and cleaving the DNAs with EcoRI. A base sequence covering the sequence GAA TTC ATG was determined by a dideoxy chain termination method

(Sanger, F. et. al., Proc. Natl. Acid. Sci. USA 74, 5463-5467, 1977), and a plasmid containing a desired sequence was selected and designated as M13mp8-hANP525.

To construct an expression plasmid, the plasmid M13mp8-hANP525 was cleaved with Eco RI and Sal I to obtain a DNA fragment of about 510 bp containing the γ -hANP gene. The Eco RI cleaves the Eco RI cleavage site incorporated by the above-mentioned in vitro mutation; and the Sal I cleaves a Sal I cleavage site originally present in M13mp8. A plasmid pS20 (Fig. 6) was cleaved with Eco RI, and Sal I, and a Eco RI - Sal I DNA fragment of about 450 bp was replaced with the above-mentioned Eco RI - Sal I fragment of about 510 bp to obtain an expression plasmid pS220 (Fig. 6). The plasmid pS20 has a λP_L promoter and SD sequence of a λ phage CII protein gene as a non-translation region followed by an Eco RI cleavage site, and can express foreign gene inserted at the Eco RI under the control of the λP_L promoter. The plasmid pS20 has been constructed by the inventor, and deposited at the Fermentation Research Institute Agency of Industrial Science and Technology (FRI) under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent (Budapest Treaty) as PERM BP-535 on May 30, 1984.

The plasmid pS220 was transformed into E. coli N4830, and the transformed strain was cultured in a minimum medium M9 supplemented with 50 μ g/ml ampicillin and 19 natural amino acids except for L-methionine at a temperature of 32°C. When the E. coli was grown to $OD_{660} = 0.4$, the temperature was shifted from 32°C to 42°C, and 35 S-methionine was added to the culture to pulse-label the newly synthesized protein for two minutes. Culturing was continued for an additional 15 minutes or 30 minutes, and terminated by the addition of trichloroacetic acid. A control culture was prepared with E. coli N4830 not containing plasmid pS220. Pro-

teins were extracted and analysed with SDS-polyacrylamido gel electrophoresis (SDS-PAGE) to determine the composition of the proteins. the culture of E. coli containing plasmid pS220 provided a band of about 20Kd which was not found in the control culture (Fig. 7). This means that the plasmid pS220 expressed a new protein with about 20Kd.

Moreover, plasmid pS223-3 and pS224-3 were constructed. The plasmid pS223-3 was constructed from plasmid pS83-3 and an Eco RI - Sal I DNA fragment of about 510 bp containing the γ -hANP gene from the M13mp8-hANP525. The plasmid pS223-3 contains an SD sequence of the lpp gene in place of the region flanked by the λP_L promotor and a translation initiation codon ATG in plasmid pS220 (Fig. 8). The plasmid pS224-3 was constructed from plasmid pS84-3 and the above-mentioned 510 bp DNA fragment. The plasmid pS224-3 contains an SD sequence of the MS2 phage A protein in place of the region flanked by the λP_L promotor and the ATC (Fig. 8). Both E. coli transformed with pS223-3 and E. coli transformed with pS224-3 produced the same proteins as those produced by E. coli transformed with pS220.

While the molecular weight (about 20Kd) shown on the SDS-PAGE is higher than a theoretical molecular weight of γ -hANP (13,679 Dalton), the protein showing about 20Kd was determined to be γ -hANP because γ -hANP prepared from human atrium cordis migrates to a site corresponding to 20Kd on the SDS-PAGE, and because the SDS-PAGE often shows a molecular weight higher than a theoretical molecular weight for other proteins.

This conclusion was confirmed as follows: a chemically synthesized linker,

5' - CTAAGTAAGTAAG
GATTCATTCATTCAGCT

wherein the underlined portions are translation termination codons, and each of the codons corresponds to any one of three kinds of reading frames, was incorporated

into a site downstream of the γ -hANP gene in a expression plasmid. The E. coli transformed with the plasmid having the three additional termination codons again produced a protein having a 20Kd molecular weight on the SDS-PAGE. This result negates the possibility that the translation termination codon TGA present at the next position to the 151st codon TAC in Fig. 5 was translated.

Thus, it was confirmed that γ -hANP, which is a precursor of α -hANP, can be produced by a microorganism using a recombinant gene according to the present invention.

Wherein, although construction of plasmids containing a base sequence coding for an amino acid sequence from 26 to 151 in Fig. 5 (γ -hANP) as a coding region are represented, other plasmids containing a base sequence coding for an amino acid sequence from 1 to 151 in Fig. 5 (signal sequence and γ -hANP), and plasmids containing a base sequence coding for an amino acid sequence from 124 to 151 (α -hANP) or any other amino acid sequence as a coding region can be constructed by the same procedure. Moreover, although cDNA corresponding to mRNA prepared from human atrium cordis was represented as a γ -hANP gene, gene coding for γ -hANP can be chemically synthesized. Moreover, although the λP_L promoter was represented as a promoter region in expression plasmids, other promoters can be used. For example, to express the gene in E. coli, a promoter region of tryptophan gene (*trp*), lactose gene (*lac*), lipoprotein gene (*lpp*), alkaline phosphatase genes (*PHOA*, *PHOS*, etc.) or outer membrane protein gene (*omp*), or a hybrid promoter of tryptophan and lactose promoters (*tac* promoter) can be used. To express the gene in yeast, a promoter region of alcohol dehydrogenase gene (*ACH*), glyceraldehyde dehydrogenase gene (*GAP-DH*), phosphoglycerokinase gene (*RGK*), or repressible acid phosphatase gene can be used. Moreover, to express the gene in animal cells, or a promoter region of SV40, an early or

late gene can be used.

3. Preparation of γ -hANP from Human Atrium Cordis

γ -hANP can be produced by the extraction of the γ -hANP from human atrium.

5 In the process, human atrium is homogenized in an acidic aqueous solution such as a phosphate buffer solution, or an acetic acid solution containing hydrochloric acid. Subsequently, γ -hANP is purified according to a conventional method suitable for the purification of peptide, such as centrifugation, isoelectric point precipitation, solvent extraction, ultrafiltration, gel filtration, adsorption chromatography or high performance liquid chromatography (HPLC), or a combination of such methods. In the above-mentioned methods, chicken rectum relaxation activity is conveniently used to select fractions containing γ -hANP, because γ -hANP has this activity. In the chromatography methods, the α -hANP containing fractions can be also selected by molecular weight (about 14,000).

20 4. Structure and Physico-chemical Properties of γ -hANP

1) Structure:

The γ -hANP has the structure set forth in Fig. 1.

25 2) Molecular weight: about 13,000 as determined by gel-filtration and about 20,000 as determined by SDS-PAGE (13679 as calculated).

3) UV spectrum: Max = 276 nm.

30 4) Color reactions: Ehrlich's reaction, negative; Sakaguchi's reaction, positive; Pauly's reaction, positive.

5) Distinction of basic, acidic or neutral property: basic.

35 6) Solubility in solvents: soluble in water, methanol, and acetic acid; insoluble in ethyl acetate, butyl acetate, ethyl ether, hexane, petroleum ether, benzene, and chloroform.

7) Amino acid composition by amino acid analysis:

Amino acid	Mol. ratio	
	found	calculated
Asx	13.69	14
Ala	11.98	11
Arg	10.24	10
Ile	1.45	1
Gly	11.61	11
Glx	13.22	12
(Cys) ₂	0.16	1
Ser	12.37	13
Tyr	2.01	2
Phe	3.48	3
Met	3.61	4
Leu	17.22	16
Val	5.98	6
Thr	2.68	2
His	1.43	1
Lys	5.14	4

25 8) Formation of salts: the γ -hANP is a basic compound as described in item 5), and can form acid addition salts with an inorganic acid such as hydrochloric acid, sulfuric acid, phosphoric acid, or an organic acid such as formic acid, acetic acid, propionic acid, succinic acid, and citric acid.

30 5. Physiological Properties of γ -hANP

The γ -hANP according to the present invention has notable diuretic, and hypotensive or antihypertensive actions.

35 Test method:

Male rats weighing 300 to 400 grams were anesthetized by intraperitoneal administration of

pentobarbital at a dosage of 60 mg/kg, and used for tests on the γ -hANP according to the method described in Life Sciences, Vol. 28, pp89-94, 1981, A.J. deBold, et.al.

5 To keep the respiratory tract open, a tracheal cannula (PE-240 Clay-Adams) was inserted into the trachea. An arterial cannula (PE-50) was inserted into a femoral artery for measurement of the blood pressure, and a venous cannula was inserted into a
10 femoral vein for the administration of Ringer's solution. 1.2 ml of Ringer's solution was injected for ten minutes, and subsequently, was constantly infused at a flow rate of 1.2 ml/hour.

 A bladder cannula made of silastic tube
15 with a inner diameter of 0.02 inches and an outer diameter of 0.037 inches was inserted into the bladder, and via the cannula, a urine sample was collected into a test tube. The collection of urine was carried out for 30 minutes before administration of the test compound, and 5, 10, 20, 30, and 60 minutes after the administration.
20

 1 nmole of the test compound γ -hANP was dissolved in 50 μ l of sterilized physiological saline with 5 μ g of bacitracin, and the solution was injected
25 into the jugular vein.

 As shown in Fig. 11, γ -hANP shows a notable diuretic action. Moreover, as shown in Fig. 12, after 1 mole of γ -hANP is administered to a rat, the blood pressure is gradually lowered by about 20 mmHg for
30 about 1 hour, revealing that γ -hANP has a hypotensive action or antihypertensive action, and may be useful as a hypotensor.

6. Use of γ -hANP as a pharmaceutical product
 Repeated administration of γ -hANP does not
35 stimulate production of antibodies, and does not cause anaphylaxis shock. γ -hANP consisting of L-amino acids is gradually hydrolized in a body providing the L-amino

acids, and therefore shows little toxicity.

Due to the higher diuretic, and blood pressure-lowering or antihypertensive actions, and the lower toxicity, γ -hANP is useful as an active ingredient for pharmaceutical compositions such as a diuretic and a hypotensor. γ -hANP is administered at 1 μ g/kg to 10 mg/kg, preferably 10 μ g/kg to 1 mg/kg.

γ -hANP can be administered in the same manner as conventional peptide type pharmaceuticals. Namely, γ -hANP is preferably administered parenterally, for example, intravenously, intramuscularly, intraperitoneally, or subcutaneously. γ -hANP, when administered orally, may be proteolytically hydrolyzed. Therefore, oral application is not usually effective. However, γ -hANP can be administered orally as a formulation wherein γ -hANP is not easily hydrolyzed in a digestive tract, such as liposome-microcapsules. γ -hANP may be also administered in suppositories, sublingual tablets, or intranasal spray.

The parenterally administered pharmaceutical composition is an aqueous solution containing about 0.000005 to 5%, preferably 0.00005 to 0.5% of γ -hANP, which may contain, in addition to γ -hANP as an active ingredient, for example, buffers such as phosphate, acetate, etc., osmotic pressure-adjusting agents such as sodium chloride, sucrose, and sorbitol, etc., antioxidative or antioxygenic agents, such as ascorbic acid or tocopherol, and preservatives, such as antibiotics. The parenterally administered composition also may be a solution readily usable or in a lyophilized form which is dissolved in sterile water before administration.

Examples

The present invention will now be further illustrated by, but is by no means limited to, the following examples.

Example 1.

1) Preparation of cDNA library, and isolation and identification of α -hANP gene

(1-a) Preparation of cDNA library

From two human atrium cordis obtained
5 from an 82 years old female and 61 years old male, 1 mg
of RNA was extracted with 4M guanidium thiocyanate
according to a method of Chirgwin et al. (Chirgwin, J.M.
et. al., Biochemistry 18, 5294-5299, 1979). The RNA was
then subjected to an oligo (dT) cellulose column using
10 10 mM Tris-HCl buffer, pH 7.2, containing 0.5M LiCl,
10 mM EDTA and 0.5% SDS as a binding buffer, and 75 μ g
of poly (A)⁺ RNA (mRNA) was isolated (Nakazato, H. &
Edmonds, D.S., Meth. Enzym. 29, 431-443, 1974). 15 μ g
of the poly (A)⁺ RNA and 4.2 μ g of vector primer DNA
15 were used to prepare a cDNA library (plasmids) according
to the Okayama-Berg method (Mol. Cell Biol. 2, 161-170,
198), and the cDNA library was used to transform E. coli
WA802. The transformants were screened on an LB-agar
medium supplemented with 40 γ /ml of ampicillin, and
20 about 40,000 colonies of ampicillin resistant trans-
formants were obtained per microgram of starting mRNA.

(1-b) Isolation of α -hANP clone

About 40,000 colonies were replicated
on a nitrocellulose filter, and the filter was incubated
25 on an LB agar plate supplemented with 40 γ /ml of
ampicillin at 37°C for 6 hours. The filter was trans-
ferred onto an LB agar plate supplemented with 180 γ /ml
of chloramphenicol, and incubated at 37°C over night.
The colonies on the filter were lysated with 0.5N NaOH,
30 and neutralized to pH 7.0, and the filter was soaked in
0.5 M Tris -HCl buffer, pH 7.0, containing 1.5 N NaCl,
and in 3 x SCC (0.15 M NaCl, 0.05M sodium citrate) for
5 minutes respectively. Finally, cell debris on the
filter was removed with a paper towel, and the filter
35 was air-dried and then baked at 80°C for 2 hours. The
filters were then subjected to hybridization with a
mixture of probes I and II (Fig. 9) consisting of

chemically synthesized 14-mer oligonucleotides labeled with ^{32}P at their 5'-end (Grunstein, M. & Hogness, D.S., Proc. Natl. Acad. Sci. USA, 72, 3961-3965, 1975). The 14-mer oligonucleotides used as a probe are possibly
5 complementary with mRNA coding for an amino acid sequence Met-Asp-Arg-Ile-Gly (an amino acid sequence from 135 to 139 in Fig. 5), and have been labeled with ^{32}P at their 5'-end using ^{32}P γ -ATP and T4 kinase and have a specific activity of 1 to 3×10^6 cpm/p mole. The
10 hybridization was carried out in 3 x SCC containing 1 x Denhardt's (0.2% BSA, Armour Pharmaceutical Company; 0.2% Ficoll, Sigma; and 0.2% polyvinyl pyrrolidone, Wako Jyunyaku), 0.1% SDS and 50 $\mu\text{g}/\text{ml}$ salmon testis DNA, at 38°C for 16 hours. The filter was then washed with
15 3 x SCC containing 0.1% SDS, air-dried, and placed in contact with an X-ray film. As a result, 85 positive clones were observed on the film. The 85 positive clones were then subjected to the colony hybridization using the same procedure as described above, except that
20 the probe I and probe II were separately used at 40°C and 38°C for each probe. As a result, 23 clones were obtained which hybridize with the probe II but do not hybridize with the probe I. Among these 23 clones 8 clones were used to isolate plasmid DNA according to a
25 conventional method. The isolated plasmid DNAs were sequenced using the probe II as the primer according to a dideoxy chain termination method (Sanger F. et al, Proc. Natl. Acad. Sci. USA, 74, 4563-5467, 1977). As a result, all of the plasmids contained a base sequence
30 corresponding to a part of an amino acid sequence of α -hANP and, consequently, the above-mentioned 8 clones were confirmed to have a plasmid containing cDNA of α -hANP. Among the 8 plasmids, 2 plasmids having a longer insert containing cDNA of γ -hANP were selected,
35 and designated as phANP 1 and phANP82. The inserts of plasmids phANP1 and phANP82 were sequenced. As a result, the plasmids phANP1 and phANP82 contained an

insert of about 950bp and an insert of about 850bp, respectively.

Restriction enzyme sites of the insert DNA region and a strategy for sequencing the insert DNA region are set forth in Fig. 10. In Fig. 10, the direction for sequencing is shown by arrows, wherein solid lines show an upper strand and dotted lines show a lower strand. The sequencing was carried out according to the Maxam-Gilbert method (Meth. Enzym., 65, 499-560, 1980) and Sanger method (Proc. Natl. Acad. Sci. USA, 74, 5463-5467, 1977). A shows the sequencing according to the Maxam-Gilbert method; B shows the sequencing according to the Sanger method; and C shows the sequencing wherein each plasmid phANP1 and phANP82 was cleaved with PstI and PvuII to obtain 3 fragments, and then each fragment was inserted into plasmid pUC8 (Vieira, J. & Messing, J., Gene 19, 259-268, 1982) and sequenced according to the Sanger method.

As a result of the above-mentioned sequencing, it was confirmed that the base sequences of inserts present in the plasmids phANP1 and phANP82 were identical except that the insert in the plasmid phANP1 has an additional base sequence of about 100bp as the 5' non-translation region. Figure 5 shows the base sequence of the insert in phANP82 and an amino acid sequence corresponding to an open reading frame in the insert. The insert DNA contains the reading frame starting from ATG and ending at a translation termination codon TGA, and the reading frame includes a base sequence coding for α -hANP at the 3' terminal side (C terminal side in the amino acid sequence). On the basis of an amino acid sequence of γ -hANP purified from human atrium cordis and identified, which has an N terminal sequence H-Asn-Pro-Met-Tyr-Asn- ... and C terminal sequence ... -Asn-Ser-Phe-Arg-Tyr-OH as set forth in Fig. 1, the reading frame was confirmed to contain a coding region of from codon AAT of Asn at 26 to codon ATC of Tyr at

151 in Fig. 5.

In Fig. 5, an amino acid sequence of from Met at 1 to Ala at 25 is considered from its characteristic sequence to be a signal sequence participating in the secretion of peptide. The base sequence in Fig. 5 also contains a sequence AATAAA shown by a solid underlining, which sequence is known to precede polyadenylation site in many eukaryotic mRNA.

2) Construction of γ -hANP gene expression vector

(2-a) Insertion of γ -hANP gene into M13 DNA

0.44 μ g of M13mp8 RF-DNA was cleaved with 16 units of PstI in 20 μ l of Medium-Salt Buffer (10 mM Tris-HCl buffer, pH 7.5, containing 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT) at 37°C for 1 hour. The mixture was then heated at 65°C for 10 minutes to stop the enzyme reaction. On the other hand, 20 μ g of plasmid pI DNA was cleaved with 160 units of PstI in 100 μ l of the Medium Salt Buffer at 37°C for 1 hour, and the reaction mixture was subjected to 1 % agarose gel electrophoresis. A part of the gel containing an DNA fragment corresponding to about 700bp was cut to obtain an agarose piece, and the DNA fragment was extracted by the electro-elution method and purified.

66 ng of the DNA fragment from M13mp8 RF-DNA and 1 μ g of the 700 bp DNA fragment were ligated using 5.6 units of T4 DNA ligase (Takara Shuzo, Japan) in 20 μ l of ligation buffer (20 mM Tris-HCl buffer, pH 7.6, containing 10 mM MgCl₂, 1 mM ATP, 5 mM DTT) at 14°C for 16 hours. E. coli JM103 cells were treated with CaCl₂ according to a conventional method to obtain a suspension of the E. coli cells in 50 mM CaCl₂, and the ligation mixture prepared as above (20 μ l) was added to the E. coli suspension to transform the E. coli.

The transformant clones were screened as follows. The suspension containing transformant E. coli cells was diluted in YT soft agar medium contain-

ing X-Gal and IPTG (prepared by adding 10 μ l of 10 mM IPTG, 50 μ l of 2% X-gal and 0.2 ml of E. coli JM103 suspension grown in a logarithmic growth phase into 3 ml of solution containing 0.6% agar, 0.8% Bacto trypton, 0.5% yeast extract and 0.5% NaCl). 0.3 ml of the diluted suspension was spread on YT agar medium (1.5% agar, 0.8% Bacto trypton, 0.5% yeast extract and 0.5% NaCl), and incubated at 37°C for 16 hours to form plaques. Among the plaques, 10 plaques were selected, and inoculated into 2 x YT liquid medium (1.6% Bacto trypton, 1% yeast extract and 1.0% NaCl) and cultured at 37°C for 8 hours. 1 ml of the cultured medium was centrifuged at 10,000 rpm for 10 minutes to recover a supernatant containing phage. The phage DNA (single strand (DNA) was isolated and purified as follows.

To 800 μ l of the phase liquid, 200 μ l of 20% polyethylen glycol (PEG 6000) containing 2.5N NaCl was added, and the mixture was allowed to stand at room temperature for 20 minutes and centrifuged at 10,000 rpm for 5 minutes to precipitate the phage. The precipitated phage was dissolved in 100 μ l of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), and to the solution 50 μ l of phenol saturated with water was added, the mixture was vigorously stirred for 5 minutes, and centrifuged at 10,000 rpm for 5 minutes. After sampling 80 μ l of the aqueous phase, to the aqueous phase 3 μ l of 3M sodium acetate, pH 8.0, and 200 μ l of ethanol was added, and the mixture was cooled at -70°C for 10 minutes, and then centrifuged at 10,000 rpm for 10 minutes to precipitate DNA. The precipitated DNA was washed once with ethanol, and dissolved in 50 μ l of the above-mentioned TE buffer. A part of a base sequence of each phage DNA was sequenced according to the dideoxy chain termination method (Methods in Enzymology, 65, 560-580, 1980, Academic Press, New York), and among 10 clones, 2 clones containing a lower strand (DNA fragment having a base sequence complementary to mRNA)

were selected. The phage DNA thus obtained were used as a template for in vitro mutation.

(2-a) Incorporation of EcoRI cleavage site and translation initiation codon by in-vitro mutation

5 To 5 μ l of the single strand phage DNA solution described above, 1 μ l of 0.2M Tris-HCl buffer, pH 7.5, containing 0.1M $MgCl_2$ and 0.5M NaCl, and 2 μ l of water, were added 2 μ l of solution containing 10 pmole of 36-mer chemically synthesized DNA fragment

10 (5'-CTCCTAGGTCAGGAATTCATGAATCCCATGTACAAT-3') phosphorylated at the 5' end to form 10 μ l of a mixture. The mixture was heated at 65°C for 5 minutes, and allowed to stand for 10 minutes at room temperature.

To the mixture, 1 μ l of 0.2 M Tris-HCl
15 buffer (pH7.5) containing 0.1 M $MgCl_2$, 2 μ l of 0.1 M DTT, 1 μ l of 10 mM ATP, 2 μ l each of 10 mM dATP, dTTP, dCTP and dTTP, 2 μ l of water, 5 units of DNA polymerase I Klenow fragment (Boehringer Mannheim) and 2.8 units of T4 DNA ligase (Takara Shuzo) were added, and the mixture
20 was incubated at 15°C for 16 hours. 20 μ l of the reaction mixture was used to transform E. coli JM103.

As described above for the preparation of phage DNA, plaques were formed on a YT soft agar medium, and 48 clones were selected. The clones were
25 inoculated to 2 x YT medium, and cultured at 37°C for 8 hours. 1 ml of the cultured medium was centrifuged at 10,000 rpm for 10 minutes to recover the supernatant as phage solution. On the other hand, RF-DNA was extracted and isolated from the precipitated cells according to
30 the alkaline extraction method (Birnboim, H.C. & Doly, J., Nucl. Acid. Res., 7, 1513-1523, 1979). The RF-DNA was then cleaved with 4.2 units of EcoRI (Takara Shuzo) in EcoRI buffer (100 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$) at 37°C for 1 hour. The reaction mixture
35 was subjected to 2% agarose gel electrophoresis, and a clone providing a DNA fragment of about 530bp was selected. The clone was designated as M13mp8-hANP525.

To 400 ml of 2 X YT liquid medium, 0.4 ml of culture of E. coli JM103 infected with the above-mentioned phage clone and 4 ml of a not-infected culture of E. coli JM103 were inoculated. The medium
5 was then incubated at 37°C for 12 hours. The cultured medium was centrifuged to obtain an infected cell precipitation and a supernatant phage solution. From the infected cells, RF-DNA was obtained by a density-gradient centrifugation method using cesium chloride and
10 ethidium bromide according to a conventional method. On the other hand, from the supernatant phage solution phage DNA was obtained, and the phage DNA was sequenced according to the dideoxy chain termination method. As a result, it was confirmed that a translation initiation
15 codon ATG and Eco RI cleavage recognition site GAATTC, i.e., a base sequence GAATTC ATG, were inserted immediately upstream of the γ -hANP gene.

(2-c) Construction of expression vector for γ -hANP gene (Fig. 6, Fig. 8)

20 An expression plasmid pS220 was constructed from plasmid pS20 and M13mp8-hANP525. The starting plasmid pS20 was constructed by the inventors, and E. coli N4380/pS20 containing the plasmid pS20 was designated as SBM271, and deposited as FERM BP-535 as
25 described above. The plasmid pS20 contains λ phage P_L promotor, and can express a foreign gene inserted to a site downstream of the promotor under the control of the promotor.

The plasmid pS20 was constructed as
30 follows. Bacteriophage λ C1857 DNA was cleaved with BamHI and Hind III to obtain a 2.4 kb DNA fragment containing the λP_L promotor. The DNA fragment was inserted into a region of Hind III-Bam HI in pBR322 to obtain a plasmid which is substantially the same as the
35 plasmid pKO 30 described in Nature 292, 128,1981. To the Hpa I cleavage site of the plasmid thus obtained, 1.3 Kb Hae III DNA fragment containing NutR, tr_1 , CII

and a part of O protein derived from bacteriophage
λcy3048 (from Dr. Hiroyuki Shimatake, Medical Department,
Toho University) was inserted to obtain plasmid (pS9),
wherein CII is present in the direction the same as the
transcription direction of λP_L . The plasmid (pS9) was
cleaved with Bgl II and Rsa I to obtain a 0.65 Kb DNA
fragment containing PL promotor, DNA sequence of pro-
tein N' which is a part of an N protein lacking a C
terminal, and the Shine-Dalgarno sequence (SD) of a CII
gene. The Rsa I and of the 0.65 Kb DNA fragment was
added with the Eco RI linker:

-CGGAATTCCG-

-GCCTTAAGGC-

(New England Biolabos, Inc.), and then the Bgl II end of
the same DNA fragment was converted to a blunt end with
T4 DNA polymerase. The DNA fragment thus obtained was
ligated with a DNA fragment prepared by Eco RI cleavage
of plasmid pBR322 and conversion of the pBR322 ends to
blunt ends to form a plasmid (pS13). In the plasmid
(pS13), the P_L promotor is oriented in the direction
the same as the transcription direction of the tetra-
cycline resistant gene (Tc^R) derived from pBR322. The
plasmid (pS13) was cleaved with Eco RI and Sal I, and a
large fragment was isolated. The large fragment was
then ligated with a DNA fragment containing a foreign
gene, i.e., human γ -interferon gene GIF, which fragment
was prepared by cleavage of plasmid pGIF4 with Eco RI
and Sal I. The plasmid pGIF4 was disclosed in Japanese
Unexamined Patent Publication No. 58-201995 (USP. Serial
No. 496176), and E. coli containing the plasmid was
designated as SBMG 105 and deposited at the FRA under
the Budapest Treaty on the International Recognition of
the Deposit of Microorganisms for the Purpose of Patent
Procedure as FERM BP-282 on May 6, 1982.

Starting with the plasmid pS20 and
M13mp8-hANP525, the γ -hANP expression vector pS220 was
constructed as follows. To 70 μ l of Eco RI reaction

buffer (100 mM Tris-HCl, pH 7.3, 50 mM NaCl, 5 mM MgCl₂) containing 8 µg of M13mp8-hANP525 DNA, 15 units of Eco RI and 15 units of Sal I were added, the reaction mixture was incubated at 37°C for 1 hour, and then

5 subjected to the 1.0% agar gel electrophoresis. About 510 bp Eco RI - Sal I DNA fraction containing γ-hANP gene was extracted from the agar gel and purified by the electroelution method described above. On the other

10 hand, to 50 µl of Eco RI reaction buffer containing 2 µg of Plasmid pS20 DNA, 10 units of Eco RI and 10 units of Sal I were added, and the reaction mixture was incubated at 37°C for 1 hour, and then subjected to 1.0% agar gel electrophoresis. According to the above-mentioned electroelution, about 4.3 Kb Eco RI - Sal I DNA fragment

15 containing the λP_L promoter and the SD sequence of the CII gene was obtained. 3 µl of an aqueous solution of the Eco RI - Sal I DNA fragment containing γ-hANP gene and 10 µl of an aqueous solution of the Eco RI - Sal I DNA fragment derived from plasmid pS20 were mixed. To

20 the mixture, 3 µl of ligation buffer with 10 times the concentration described in section (2-a), 3 µl of 100 mM DTT, 3 µl of 1 mM ATP aqueous solution, 6 µl of distilled water, and 2 units of T4 DNA ligase were added, and the mixture was incubated at 16°C overnight

25 to obtain a ligation mixture. The ligation mixture was used to transform E. coli N4830 (from Pharmacia P-L Biochemicals) as follows. The E. coli cells were treated according to a conventional procedure to obtain competent cells. To 300 µl of the treated E. coli cell

30 suspension containing 50 mM CaCl₂, 10 µl of the ligation mixture obtained as above was added, and the resulting mixture was allowed to stand for 1 hour in ice. 2.5 µl of L-broth was added to the mixture, which was then incubated at 32°C for 1.5 hours. The

35 resulting mixture was plated on a nutrient agar medium containing 50 µg/ml of ampicillin to select ampicillin resistant colonies which are transformants. The trans-

formants were treated according to a conventional procedure to isolate plasmid DNAs. The plasmids were screened by analysis using restriction enzymes. A plasmid having the γ -hANP gene downstream of the λP_L promoter was selected, and designated as pS220. The transformant containing the plasmid pS220 was designated as E. coli N4380/pS220, and was used in the test for γ -hANP production as described below in detail.

In addition to the γ -hANP gene expression vector pS220, other γ -hANP gene expression vectors were constructed as follows. Plasmid pS20 (Fig. 6) was cleaved with Eco RI, and hydrolyzed with an exonuclease Bal 31 to form DNA fragments having various lengths. The DNA fragments were ligated with Xba I linker (from New England Biolabos Inc.; dCTCTAGAG) to obtain plasmid pS20X. The plasmid pS20X was cleaved with Xba I and Sal I to delete any Xba I - Sal I short fragments. On the other hand, plasmid pIN4GIF54 was cleaved with Xba I and Sal I to obtain a short Xba I - Sal I fragment containing human γ -interferon gene, which short fragment was then inserted to the above-mentioned cleaved pS20X in place of the deleted Xba I - Sal I fragment to form plasmids. Among the plasmids thus formed, a plasmid which can effectively express the human γ -interferon gene when transformed into E. coli was designated as pS83-3. The above-mentioned plasmid pIN4GIF54 and a method for measuring an amount of γ -interferon were disclosed in Japanese Unexamined Patent Publication No. 60-24187 (USP. Serial No. 632204). As shown in Fig. 8, plasmid pS83-3 was cleaved with Eco RI and Sal I to delete the Eco RI - Sal I short fragment consisting of the human γ -interferon gene (GIF). The plasmid M13mp8-hANP525 was cleaved with Eco RI and Sal I to obtain an about 510bp Eco RI - Sal I fragment containing γ -hANP gene, which fragment was then inserted to the cleaved pS83-3 in place of the deleted Eco RI - Sal I fragment consisting of GIF to form plasmid pS223-3. The

plasmid pS223-3 contains an λP_L promotor region, SD sequence of E. coli lpp gene, and γ -hANP gene, in this order. E. coli N4380 transformed with the plasmid pS223-3 was designated as E. coli N4380/pS223-3, and
5 used in the test for γ -hANP production as described below in detail.

Another γ -hANP gene expression vector pS224-3 was constructed as follows. The plasmid pS83-3 was cleaved with Xba I and Eco RI to delete the SD
10 sequence of the lpp gene, and in place of the deleted SD sequence of the lpp gene, a chemically synthesized DNA fragment AGGAGGT with Xba I and Eco RI cohesive ends, which is the SD sequence of the bacteriophage MS2A protein gene, was inserted into the cleaved pS83-3 to
15 form plasmid pS84-3. As shown in Fig. 8, the plasmid pS84-3 DNA was cleaved with Eco RI and Sal I to delete the Eco RI - Sal I short fragment consisting of GIF. The plasmid M13mp8-hANP525 DNA was cleaved with Eco RI and Sal I to obtain an about 510bp Eco RI - Sal I
20 fragment containing the γ -hANP gene, which fragment was then inserted into the cleaved pS84-3 DNA in place of the deleted Eco RI - Sal I fragment consisting of GIF to obtain plasmid pS224-3. The plasmid pS224-3 thus obtained contained a λP_L promotor region, SD sequence of MS2A,
25 and γ -hANP gene, in this order. E. coli N4380 transformed with the plasmid pS224-3 was designated as E. coli N4380/pS224-3, and tested for the production of γ -hANP.

3) Production of γ -hANP by transformant

30 Three transformants, i.e., E. coli N4830/pS220, N4830/pS223-3 and N4830/pS224-3, and a control strain E. coli N4830 containing no plasmid were compared. Each of these four strains was cultured in an M9 minimum medium supplemented with 50 μ g/ml of ampicillin and
35 19 natural amino acids except for L-methionine at 32°C until the optical density at 660 nm (OD 660) of the culture reached 0.4. At this point each culture was

divided into two cultures. One of the divided cultures was cultured at the same temperature, i.e., 32°C; and the other was cultured at 42°C. At 15 minutes and 30 minutes after the division of the culture, 0.3 ml of culture was obtained from each culture, and to the culture (0.3 ml), 2 μ Ci of 35 S-methionine with 800 Ci/m mole of specific activity (New England Nuclea) was added. Culturing was continued for 2 minutes and then trichloroacetic acid was added to the concentration of 10% to each culture, which was then cooled to 0°C. The mixture was then centrifuged for 10 minutes, and the precipitate was washed once with ethanol and subjected to the 15% SDS polyacrylamide gel electrophoresis to separate proteins. The gel was dried and subjected to a radioautography to detect bands of proteins labeled with 35 S-methionine.

As shown in Fig. 7, for cultures at 42°C of three transformants, i.e., N4830/pS220, N4830/pS223-3, and N4830/pS224-3, a protein band corresponding to a molecular weight of 20Kd was found. The 20Kd band was not found for a culture at 32°C of the three transformants, and a culture at 32°C and 42°C of the control strain. This means that a protein corresponding to the 20Kd band was produced by expression of the γ -hANP gene under the λP_L promotor. The expression product is confirmed to be γ -hANP by the fact that γ -hANP prepared from human atrium cordis migrates to a site the same as the expression product on SDS polyacrylamide gel electrophoresis under the same condition. In the SDS polyacrylamide gel electrophoresis, a standard protein kit for determination of the molecular weight supplied by Pharmacia P-L Biochemicals was used.

Example 2. Preparation of γ -hANP from human atrium cordis

377 g of human atrium cordis was removed and boiled in ten volumes of 1 M acetic acid aqueous solution for 10 minutes, to inactivate protease present in the atrium

cordis. The boiled atrium cordis in the acetic acid solution was then cooled to 4°C, added with cooled acetone to two volumes and homogenized with a Polytron homogenizer to extract the γ -hANP. The homogenate thus
5 obtained was centrifuged at 16000 XG for 30 minutes to obtain a supernatant. From the supernatant, acetone was removed by vacuum distillation to obtain 150 ml of an extract containing γ -hANP.

To the extract water was added to 1% of total
10 volume, and the diluted extract was subjected to reverse column chromatography using 90 ml of LCSORB SP-C-ODS column (Chemco). The charged column was washed with 450 ml of 0.1 N acetic acid solution, and eluted with 450 ml of a mixed eluent of water: acetonitrile: 10% trifluoro-
15 acetic acid (40:60:1). The elute was fractionated to a green fraction at an early stage and a brown fraction at a later stage. 225 ml of the green fraction was then lyophilized to obtain 90 mg of dried residue.

The residue thus obtained was dissolved in 5 ml of
20 1 N acetic acid, and the solution was subjected to gel filtration using a Sephadex G-75 column (Pharmacia, 1.8 x 135 cm) equilibrated with a 1 N acetic acid solution. The elution was carried out with 1 N acetic acid, and 5 ml of fractions were obtained. The elution
25 profile is set forth in Fig. 13. Fractions No. 39 to 44 having chicken rectum relaxation activity were combined, and the combined fraction was subjected to HPLC using a TSK ODS column (Toyo Soda, 4.0 x 250 mm) and two eluents, i.e., (A) a mixture of water: acetonitrile: 10% tri-
30 fluoroacetic acid (90:10:1) and (B) a mixture of water: acetonitrile: 10% acetic acid (40:60:1). The column was equilibrated with eluent (A), and 250 μ l of the active fraction obtained from the Sephadex G-75 column was applied to the column. Elution was carried out by
35 linear gradient from (A) to (B) at a flow rate of 1.0 ml/min and at a pressure of 110 to 130 Kg/cm² for 80 minutes. The result is set forth in Fig. 14. A peak

fraction having chick rectum relaxation activity (retention time 50 minutes) was obtained, and subjected to HPLC using the same column. Elution was carried out using an eluent (A) water: acetonitrile: 10% trifluoroacetic acid (70:30:1) and an eluent (B) water: acetonitrile: 10% trifluoroacetic acid (40:60:1) in the same manner as described above. The result is set forth in Fig. 15. The main peak was fractionated to obtain about 3.37 nmole of purified γ -hANP for one run. The same procedure was repeated to obtain 404 nmole of γ -hANP.

Example 3. Preparation of parenteral composition

A) Injection solution

Composition

	γ -hANP	2 g
15	sodium chloride	8 g
	ascorbic acid	2 g
	sterile water	1 l

Method

γ -hANP and sodium chloride were dissolved in sterile water, an ampule was filled with 5 ml of the solution, and the ampule was then sealed.

B) Lyophilizate

Composition

	γ -hANP	2 g
25	sorbital	20 g

Method

γ -hANP and sorbital were dissolved in 200 ml of sterile water, a vial was filled with 1 ml of the solution, and lyophilized, and the vial was then sealed.

The composition is dissolved in 5 ml of sterile water before parenteral administration.

CLAIMS

1. A DNA fragment comprising a base sequence coding for a peptide occurring in human atrium cordis and having diuretic action or a precursor of the peptide.

2. A DNA fragment according to claim 1, wherein
5 the base sequence codes for a peptide consisting of all or a part of the following amino acid sequence:

Met Ser Ser Phe Ser Thr Thr Thr Val Ser
Phe Leu Leu Leu Leu Ala Phe Gln Leu Leu
Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn
10 Ala Val Ser Asn Ala Asp Leu Met Asp Phe
Lys Asn Leu Leu Asp His Leu Glu Glu Lys
Met Pro Leu Glu Asp Glu Val Val Pro Pro
Gln Val Leu Ser Glu Pro Asn Glu Glu Ala
Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
15 Pro Pro Trp Thr Gly Glu Val Ser Pro Ala
Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly
Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu
Leu Lys Ser Lys Leu Arg Ala Leu Leu Thr
Ala Pro Arg Ser Leu Arg Arg Ser Ser Cys
20 Phe Gly Gly Arg Met Asp Arg Ile Gly Ala
Gln Ser Gly Leu Gly Cys Asn Ser Phe Arg
Tyr.

3. A DNA fragment according to claim 2, wherein the peptide has the following amino acid sequence:

25 Asn Pro Met Tyr Asn Ala Val Ser Asn Ala
Asp Leu Met Asp Phe Lys Asn Leu Leu Asp
His Leu Glu Glu Lys Met Pro Leu Glu Asp
Glu Val Val Pro Pro Gln Val Leu Ser Glu
Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser
30 Pro Leu Pro Glu Val Pro Pro Trp Thr Gly
Glu Val Ser Pro Ala Gln Arg Asp Gly Gly
Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser
Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu
Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu
35 Arg Arg Ser Ser Cys Phe Gly Gly Arg Met
Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly

Cys Asn Ser Phe Arg Try.

4. A DNA fragment according to claim 2, wherein the base sequence consists of all or a part of the following sequence:

```

5       ATG AGC TCC TTC TCC ACC ACC ACC GTG AGC
      TTC CTC CTT TTA CTG GCA TTC CAG CTC CTA
      GGT CAG ACC AGA GCT AAT CCC ATG TAC AAT
      GCC GTG TCC AAC GCA GAC CTG ATG GAT TTC
      AAG AAT TTG CTG GAC CAT TTG GAA GAA AAG
10     ATG CCT TTA GAA GAT GAG GTC GTG CCC CCA
      CAA GTG CTC AGT GAG CCG AAT GAA GAA GCG
      GGG GCT GCT CTC AGC CCC CTC CCT GAG GTG
      CCT CCC TGG ACC GGG GAA GTC AGC CCA GCC
      CAG AGA GAT GGA GGT GCC CTC GGG CGG GGC
15     CCC TGG GAC TCC TCT GAT CGA TCT GCC CTC
      CTA AAA AGC AAG CTG AGG GCG CTG CTC ACT
      GCC CCT CGG AGC CTG CGG AGA TCC AGC TGC
      TTC GGG GGC AGG ATG GAC AGG ATT GGA GCC
      CAG AGC GGA CTG GGC TGT AAC AGC TTC CGG
20     TAC.

```

5. A DNA fragment according to claim 3, wherein the peptide is coded by the following base sequence:

```

      AAT CCC ATG TAC AAT GCC GTG TCC AAC GCA
      GAC CTG ATG GAT TTC AAG AAT TTG CTG GAC
25     CAT TTG GAA GAA AAG ATG CCT TTA GAA GAT
      GAG GTC GTG CCC CCA CAA GTG CTC AGT GAC
      CCG AAT GAA GAA GCG GGG GCT GCT CTC AGC
      CCC CTC CCT GAG GTG CCT CCC TGG ACC GGG
      GAA GTC AGC CCA GCC CAG AGA GAT GGA GGT
30     GCC CTC GGG CGG GGC CCC TGG GAC TCC TCT
      GAT CGA TCT GCC CTC CTA AAA AGC AAG CTG
      AGG GCG CTG CTC ACT GCC CCT CGG AGC CTG
      CGG AGA TCC AGC TGC TTC GGG GGC AGG ATG
      GAC AGG ATT GGA GGC CAG AGC GGA CTG GGC
35     TGT AAC AGC TTC CGG TAC.

```

6. A DNA fragment according to any one of the preceding claims, wherein the DNA fragment is

cDNA prepared by using mRNA as a template, which mRNA is obtained from human atrium cordis.

7. A plasmid containing a promotor region, SD sequence, and a DNA fragment comprising a base sequence
5 coding for a peptide occurring in human atrium cordis and having diuretic action or a precursor of the peptide.

8. A plasmid according to claim 7, wherein the peptide consists of all or a part of the following amino acid sequence:

10 Met Ser Ser Phe Ser Thr Thr Thr Val Ser
Phe Leu Leu Leu Leu Ala Phe Gln Leu Leu
Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn
Ala Val Ser Asn Ala Asp Leu Met Asp Phe
Lys Asn Leu Leu Asp His Leu Glu Glu Lys
15 Met Pro Leu Glu Asp Glu Val Val Pro Pro
Gln Val Leu Ser Glu Pro Asn Glu Glu Ala
Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
Pro Pro Trp Thr Gly Glu Val Ser Pro Ala
Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly
20 Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu
Leu Lys Ser Lys Leu Arg Ala Leu Leu Thr
Ala Pro Arg Ser Leu Arg Arg Ser Ser Cys
Phe Gly Gly Arg Met Asp Arg Ile Gly Ala
Gln Ser Gly Leu Gly Cys Asn Ser Phe Arg
25 Tyr.

9 A plasmid according to claim 8, wherein the peptide consists of the following amino acid sequence:

Asn Pro Met Tyr Asn Ala Val Ser Asn Ala
Asp Leu Met Asp Phe Lys Asn Leu Leu Asp
30 His Leu Glu Glu Lys Met Pro Leu Glu Asp
Glu Val Val Pro Pro Gln Val Leu Ser Glu
Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser
Pro Leu Pro Glu Val Pro Pro Trp Thr Gly
Glu Val Ser Pro Ala Gln Arg Asp Gly Gly
35 Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser
Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu
Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu

Arg Arg Ser Ser Cys Phe Gly Gly Arg Met
Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly
Cys Asn Ser Phe Arg Tyr.

5 10. A plasmid according to claim 8, wherein the
amino acid sequence is coded by the following base
sequence:

ATG AGC TCC TTC TCC ACC ACC ACC GTG AGC
TTC CTC CTT TTA CTG GCA TTC CAG CTC CTA
GGT CAG ACC AGA GCT AAT CCC ATG TAC AAT
10 GCC GTG TCC AAC GCA GAC CTG ATG GAT TTC
AAG AAT TTG CTG GAC CAT TTG GAA GAA AAG
ATG CCT TTA GAA GAT GAG GTC GTG CCC CCA
CAA GTG CTC AGT GAG CCG AAT GAA GAA GCG
GGG GCT GCT CTC AGC CCC CTC CCT GAG GTG
15 CCT CCC TGG ACC GGG GAA GTC AGC CCA GCC
CAG AGA GAT GGA GGT GCC CTC GGG CGG GGC
CCC TGG GAC TCC TCT GAT CGA TCT GCC CTC
CTA AAA AGC AAG CTG AGG GCG CTG CTC ACT
GCC CCT CGG AGC CTG CGG AGA TCC AGC TGC
20 TTC GGG GGC AGG ATG GAC AGG ATT GGA GCC
CAG AGC GGA CTG GGC TGT AAC AGC TTC CGG
TAC.

25 11. A plasmid according to claim 9, wherein the
amino acid sequence is coded by the following base
sequence:

AAT CCC ATG TAC AAT GCC GTG TCC AAC GCA
GAC CTG ATG GAT TTC AAG AAT TTG CTG GAC
CAT TTG GAA GAA AAG ATG CCT TTA GAA GAT
GAG GTC GTG CCC CCA CAA GTG CTC AGT GAC
30 CCG AAT GAA GAA GCG GGG GCT GCT CTC AGC
CCC CTC CCT GAG GTG CCT CCC TGG ACC GGG
GAA GTC AGC CCA GCC CAG AGA GAT GGA GGT
GCC CTC GGG CGG GGC CCC TGG GAC TCC TCT
GAT CGA TCT GCC CTC CTA AAA AGC AAG CTG
35 AGG GCG CTG CTC ACT GCC CCT CGG AGC CTG
CGG AGA TCC AGC TGC TTC GGG GGC AGG ATG
GAC AGG ATT GGA GCC CAG AGC GGA CTG GGC

TGT AAC AGC TTC CGG TAC.

12. A plasmid according to any one of claims 7 to 11 wherein the promotor is a λP_L promotor.

5 13. A plasmid according to any one of claims 7 to 11 wherein the SD sequence is an SD sequence of a CII gene, SD sequence of an lpp gene or SD sequence of an MS2A gene.

14. A plasmid according to claim 7, wherein the plasmid is pS220, pS223-3, or pS224-3.

10 15. A microorganism transformed with a plasmid containing a promotor region, SD sequence, and a DNA fragment comprising a base sequence coding for a peptide occurring in human atrium cordis and having diuretic action or a precursor of the peptide.

15 16. A microorganism according to claim 15, wherein the peptide consists of all or a part of the following amino acid sequence:

Met Ser Ser Phe Ser Thr Thr Thr Val Ser
Phe Leu Leu Leu Leu Ala Phe Gln Leu Leu
20 Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn
Ala Val Ser Asn Ala Asp Leu Met Asp Phe
Lys Asn Leu Leu Asp His Leu Glu Glu Lys
Met Pro Leu Glu Asp Glu Val Val Pro Pro
Gln Val Leu Ser Glu Pro Asn Glu Glu Ala
25 Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
Pro Pro Trp Thr Gly Glu Val Ser Pro Ala
Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly
Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu
Leu Lys Ser Lys Leu Arg Ala Leu Leu Thr
30 Ala Pro Arg Ser Leu Arg Arg Ser Ser Cys
Phe Gly Gly Arg Met Asp Arg Ile Gly Ala
Gln Ser Gly Leu Gly Cys Asn Ser Phe Arg
Tyr.

35 17. A microorganism according to claim 16, wherein the peptide consists of the following amino acid sequence:

Asn Pro Met Tyr Asn Ala Val Ser Asn Ala
Asp Leu Met Asp Ph Lys Asn Leu Leu Asp

5 His Leu Glu Glu Lys Met Pro Leu Glu Asp
 Glu Val Val Pro Pro Gln Val Leu Ser Glu
 Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser
 Pro Leu Pro Glu Val Pro Pro Trp Thr Gly
 Glu Val Ser Pro Ala Gln Arg Asp Gly Gly
 Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser
 Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu
 Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu
 Arg Arg Ser Ser Cys Phe Gly Gly Arg Met
 10 Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly
 Cys Asn Ser Phe Arg Tyr.

18. A microorganism according to claim 16, wherein the amino acid sequence is coded by the following base sequence:

15 ATG AGC TCC TTC TCC ACC ACC ACC GTG AGC
 TTC CTC CTT TTA CTG GCA TTC CAG CTC CTA
 GGT CAG ACC AGA GCT AAT CCC ATG TAC AAT
 GCC GTG TCC AAC GCA GAC CTG ATG GAT TTC
 AAG AAT TTG CTG GAC CAT TTG GAA GAA AAG
 20 ATG CCT TTA GAA GAT GAG GTC GTG CCC CCA
 CAA GTG CTC AGT GAG CCG AAT GAA GAA GCG
 GGG GCT GCT CTC AGC CCC CTC CCT GAG GTG
 CCT CCC TGG ACC GGG GAA GTC AGC CCA GCC
 CAG AGA GAT GGA GGT GCC CTC GGG CGG GGC
 25 CCC TGG GAC TCC TCT GAT CGA TCT GCC CTC
 CTA AAA AGC AAG CTG AGG GCG CTG CTC ACT
 GCC CCT CGG AGC CTG CGG AGA TCC AGC TGC
 TTC GGG GGC AGG ATG GAC AGG ATT GGA GCC
 CAG AGC GGA CTG GGC TGT AAC AGC TTC CGG
 30 TAC.

19. A microorganism according to claim 17, wherein the amino acid sequence is coded by the following base sequence:

35 AAT CCC ATG TAC AAT GCC GTG TCC AAC GCA
 GAC CTG ATG GAT TTC AAG AAT TTG CTG GAC
 CAT TTG GAA GAA AAG ATG CCT TTA GAA GAT
 GAG GTC GTG CCC CCA CAA GTG CTC AGT GAC

5 CCG AAT GAA GAA GCG GGG GCT GCT CTC AGC
 CCC CTC CCT GAG GTG CCT CCC TGG ACC GGG
 GAA GTC AGC CCA GCC CAG AGA GAT GGA GGT
 GCC CTC GGG CGG GGC CCC TGG GAC TCC TCT
 GAT CGA TCT GCC CTC CTA AAA AGC AAG CTG
 AGG GCG CTG CTC ACT GCC CCT CGG AGC CTG
 CGG AGA TCC AGC TGC TTC GGG GGC AGG ATG
 GAC AGG ATT GGA GCC CAG AGC GGA CTG GGC
 TGT AAC AGC TTC CGG TAC.

10 20. A microorganism according to any one of claims 15 to 19 wherein the microorganism is E. coli.

15 21. A process for production of a peptide occurring in human atrium cordis and having diuretic action and/or a precursor of the peptide, comprising culturing a microorganism transformed with a plasmid containing a

20 promoter region, SD sequence, and a DNA fragment comprising a base sequence coding for the peptide, or a precursor peptide to form the respective peptide and recovering the same; or

converting such a peptide or precursor to an acid addition salt thereof; or

25 converting an acid addition salt of such a peptide or precursor to the free base form thereof.

22. A process according to claim 21, wherein the peptide has all or part of the following amino acid sequence:

30 Met Ser Ser Phe Ser Thr Thr Thr Val Ser
 Phe Leu Leu Leu Leu Ala Phe Gln Leu Leu
 Gly Gln Thr Arg Ala Asn Pro Met Try Asn
 Ala Val Ser Asn Ala Asp Leu Met Asp Phe
 Lys Asn Leu Leu Asp His Leu Glu Glu Lys
 Met Pro Leu Glu Asp Glu Val Val Pro Pro
 Gln Val Leu Ser Glu Pro Asn Glu Glu Ala
 Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
 35 Pro Pro Trp Thr Gly Glu Val Ser Pro Ala
 Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly

5 Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu
Leu Lys Ser Lys Leu Arg Ala Leu Leu Thr
Ala Pro Arg Ser Leu Arg Arg Ser Ser Cys
Phe Gly Gly Arg Met Asp Arg Ile Gly Ala
Gln Ser Gly Leu Gly Cys Asn Ser Phe Arg
Tyr.

23. A process according to claim 22, wherein the peptide has the following amino acid sequence:

10 Asn Pro Met Tyr Asn Ala Val Ser Asn Ala
Asp Leu Met Asp Phe Lys Asn Leu Leu Asp
His Leu Glu Glu Lys Met Pro Leu Glu Asp
Glu Val Val Pro Pro Gln Val Leu Ser Glu
Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser
15 Pro Leu Pro Glu Val Pro Pro Trp Thr Gly
Glu Val Ser Pro Ala Gln Arg Asp Gly Gly
Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser
Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu
Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu
Arg Arg Ser Ser Cys Phe Gly gly Arg Met
20 Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly
Cys Asn Ser Phe Arg Tyr.

24. A process according to claim 21 wherein the DNA fragment is as set forth in any of claims 2 to 6.

25 25. A precursor of a diuretic peptide γ -hANP of the following amino acid sequence:

30 Met Ser Ser Phe Ser Thr Thr Thr Val Ser
Phe Leu Leu Leu Leu Ala Phe Gln Leu Leu
Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn
Ala Val Ser Asn Ala Asp Leu Met Asp Phe
Lys Asn Leu Leu Asp His Leu Glu Glu Lys
Met Pro Leu Glu Asp Glu Val Val Pro Pro
Gln Val Leu Ser Glu Pro Asn Glu Glu Ala
Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
35 Pro Pro Trp Thr Gly Glu Val Ser Pro Ala
Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly

5 Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu
 Leu Lys Ser Lys L u Arg Ala Leu Leu Thr
 Ala Pro Arg Ser Leu Arg Arg Ser Ser Cys
 Phe Gly Gly Arg Met Asp Arg Ile Gly Ala
 Gln Ser Gly Leu Gly Cys Asn Ser Phe Arg
 Tyr,

and acid addition salt thereof.

26. A diuretic peptide γ -hANP of the following amino acid sequence:

10 Asn Pro Met Tyr Asn Ala Val Ser Asn Ala
 Asp Leu Met Asp Phe Lys Asn Leu Leu Asp
 His Leu Glu Glu Lys Met Pro Leu Glu Asp
 Glu Val Val Pro Pro Gln Val Leu Ser Glu
 Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser
 15 Pro Leu Pro Glu Val Pro Pro Trp Thr Gly
 Glu Val Ser Pro Ala Gln Arg Asp Gly Gly
 Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser
 Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu
 Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu
 20 Arg Arg Ser Ser Cys Phe Gly Gly Arg Met
 Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly
 Cys Asn Ser Phe Arg Tyr,

and acid addition salt thereof.

25 27. A diuretic composition containing a peptide
 γ -hANP according to claim 26 or an acid addition salt
 thereof with a conventional pharmaceutical additive.

28. A hypotensor composition containing a peptide
 γ -hANP according to claim 26, or an acid addition
 salt thereof with a conventional pharmaceutical additive.

30 29. A composition according to claim 27 or
 claim 28, wherein the composition is a solution for
 parenteral administration and the additive is a buffer,
 an osmotic pressure adjusting agent or a preservative,
 or a combination thereof.

30. A composition according to any one of claims 27, 28 or 29 wherein the composition is a solution for parenteral administration and contains about 0.000005 to 5% of the γ -hANP.

5 31. A composition according to any one of claims 27 to 30, wherein the composition is in a lyophilized form.

10 32. The medical use of γ -hANP as defined in claim 26, or an acid addition salt thereof, or a composition according to any one of claims 27 to 31.

Fig. 1

Asn	Pro	Met	Tyr	Asn	Ala	Val	Ser	Asn	Ala	Asp	Leu	Met	Asp	Phe	Lys	Asn	Leu	Leu	Asp	20
His	Leu	Glu	Glu	Lys	Met	Pro	Leu	Glu	Asp	Glu	Val	Val	Pro	Pro	Gln	Val	Leu	Ser	Glu	40
Pro	Asn	Glu	Glu	Ala	Gly	Ala	Ala	Leu	Ser	Pro	Leu	Pro	Glu	Val	Pro	Pro	Trp	Thr	Gly	60
Glu	Val	Ser	Pro	Ala	Gln	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	Gly	Pro	Try	Asp	Ser	Ser	80
Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser	Lys	Leu	Arg	Ala	Leu	Leu	Thr	Ala	Pro	Arg	Ser	Leu	100
Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	Met	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly	120
Cys	Asn	Ser	Phe	Arg	Tyr															

Fig. 2

1	Met	Ser	Ser	Phe	Ser	Thr	Thr	Thr	Val	Ser	Phe	Leu	Leu	Leu	Leu	Ala	Phe	Gln	Leu	Leu	20
	Gly	Gln	Thr	Arg	Ala	Asn	Pro	Met	Tyr	Asn	Ala	Val	Ser	Asn	Ala	Asp	Leu	Met	Asp	Phe	40
	Lys	Asn	Leu	Leu	Asp	His	Leu	Glu	Glu	Lys	Met	Pro	Leu	Glu	Asp	Glu	Val	Val	Pro	Pro	60
	Gln	Val	Leu	Ser	Glu	Pro	Asn	Glu	Glu	Ala	Gly	Ala	Ala	Leu	Ser	Pro	Leu	Pro	Glu	Val	80
	Pro	Pro	Trp	Thr	Gly	Glu	Val	Ser	Pro	Ala	Gln	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	Gly	100
	Pro	Trp	Asp	Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser	Lys	Leu	Arg	Ala	Leu	Leu	Thr	120
	Ala	Pro	Arg	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	Met	Asp	Arg	Ile	Gly	Ala	140
	Cln	Ser	Gly	Leu	Gly	Cys	Asn	Ser	Phe	Arg	Tyr										150

Fig. 3

10
 AAT CCC ATG TAC AAT GCC GTG TCC AAC GCA GAC CTG ATG GAT TTC AAG AAT TTG CTG GAC 20
 30
 CAT TTG GAA GAA AAG ATG CCT TTA GAA GAT GAG GTC GTG CCC CCA CAA GTG CTC AGT GAG 40
 50
 CCG AAT GAA GAA GCG GGG GCT GCT CTC AGC CCC CTC CCT GAG GTG CCT CCC TGG ACC GGG 60
 70
 GAA GTC AGC CCA GCC CAG AGA GAT GGA GGT GCC CTC GGG CGG GGC CCC TGG GAC TCC TCT 80
 90
 GAT CGA TCT GCC CTC CTA AAA AGC AAG CTG AGG GCG CTG CTC ACT GCC CCT CGG AGC CTG 100
 110
 CGG AGA TCC AGC TGC TTC TGC GGG GGC AGG ATG GAC AGG ATT GGA GCC CAG AGC GGA CTG GGC 120
 126
 TGT AAC AGC TTC CGG TAC

Fig. 4

	1	ATG AGC TCC TTC TCC ACC ACC ACC GTG AGC TTC CTC CTT TTA CTG GCA TTC CAG CTC CTA	20
	10		
	30	GGT CAG ACC AGA GCT AAT CCC ATG TAC AAT GCC GTG TCC AAC GCA GAC CTG ATG GAT TTC	40
	50	AAG AAT TTG CTG GAC CAT TTG GAA GAA AAG ATG CCT TTA GAA GAT GAG GTC GTG CCC CCA	60
	70	CAA GTG CTC AGT GAG CCG AAT GAA GAA GCG GGG GCT GCT CTC AGC CCC CTC CCT GAG GTG	80
	90	CCT CCC TGG ACC ACC GGG GAA GTC AGC CCA GCC CAG AGA GAT GGA GGT GCC CTC GGG CGG GGC	100
	110	CCC TGG GAC TCC TCT GAT CGA TCT GCC CTC CTA AAA AGC AAG CTG AGG GCG CTG CTC ACT	120
	130	GCC CCT CGG AGC CTG CGG AGA TCC AGC TGC TTC GGG GGC AGG ATG GAC AGG ATT GGA GCC	140
	150	CAG AGC GGA CTG GGC TGT AAC AGC TTC CGG TAC	

Fig. 5

Fig. 5A

Fig. 5B

Fig. 5A

CCAAGAGAGGGGAACACAGAGGGAACACAGAGGGGAGAGACAGACAGCAAGCAGTGGATTGCTCTTGACGACGCCAGC -1

1 Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Ala Phe Gln Leu Leu 20
 ATG AGC TCC TTC TCC ACC ACC ACC ACC GTG AGC TTT TTA CTG GCA TTC CAG CTC CTA 60

30 Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val Ser Asn Ala Asp Leu Met Asp Phe 40
 GGT CAG ACC AGA GCT AAT CCC ATG TAC AAT GCC GTG TCC AAC GCA GAC CTG ATG GAT TTC 120

50 Lys Asn Leu Leu Asp His Leu Glu Glu Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro 60
 AAG AAT TTG CTG GAC CAT TTG GAA GAA GAA AAG AAG ATG CCT TTA GAA GAT GAG GTC GTG CCC CCA 180

70 Gln Val Leu Ser Glu Pro Asn Glu Glu Glu Ala Ala Leu Ser Pro Leu Pro Glu Val 80
 CAA GTG CTC AGT GAG CCG AAT GAA GAA GCG GGG GCT GCT CTC AGC CCC CTC CCT GAG GTG 240

-90
 5'-ACAGACGTAGG -80

Fig. 5B

Pro	Pro	Trp	Thr	Gly	Glu	Val	Ser	Pro	Ala	Gln	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	Gly	100	
CCT	CCC	TGG	ACC	GGG	GAA	GTC	AGC	CCA	GCC	CAG	AGA	GAT	GGA	GGT	GCC	CTC	GGG	CGG	GGC	300	
Pro	Trp	Asp	Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser	Lys	Leu	Arg	Ala	Leu	Leu	Thr	120	
CCC	TGG	GAC	TCC	TCT	GAT	CGA	TCT	GCC	CTC	CTA	AAA	AGC	AAG	CTG	AGG	GCG	CTG	CTC	ACT	360	
Ala	Pro	Arg	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	Met	Asp	Arg	Ile	Gly	Ala	140	
GCC	CCT	CGG	AGC	CTG	CGG	AGA	TCC	AGC	TGC	TTC	GCG	GCG	AGG	ATG	GAC	AGG	ATT	GGA	GCC	420	
Gln	Ser	Gly	Leu	Gly	Cys	Asn	Ser	Phe	Arg	Try	TGA										487
CAG	AGC	GGA	CTG	GCG	TGT	AAC	AGC	TTC	CGG	TAC	AGATAACAGCCAGGGAGGACAAGCAGGGCTG										566
GGCCTAGGGACAGACTGCAAGAGGCTCCTGTCCCTGGGGTCTCTGCTGCATTGTGTGATCTTGTGGCCATGGAGTTG																				566	
TGATCATCCCCATCTAAGCTGCAGCTTCCTGTCAACACTTCTCACATCTTATGCTAACTGTAGATAAAAGTGGTTGATGG																				645	
TGACTTCCCTCGCCCTCTCCCAACCCCATGCATTAAATTTAAGGTAGAACCTCACCTGTACTGAAAAGTGGTTGAAAAGTG																				724	
AATAAACTTCAGCACCATGGACAGAAGAC-3'																				753	

6/15

0164273

Fig. 6

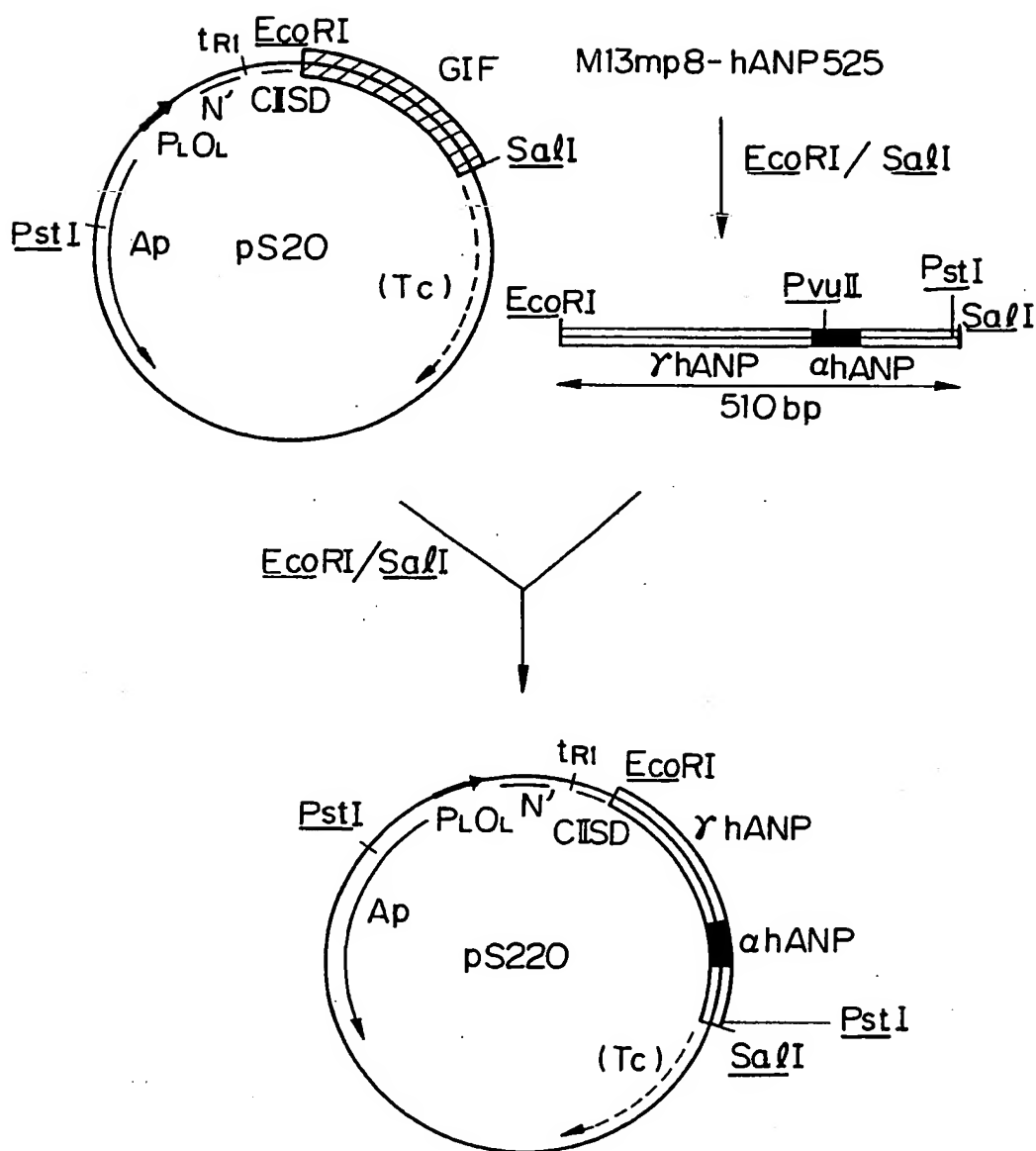


Fig. 7

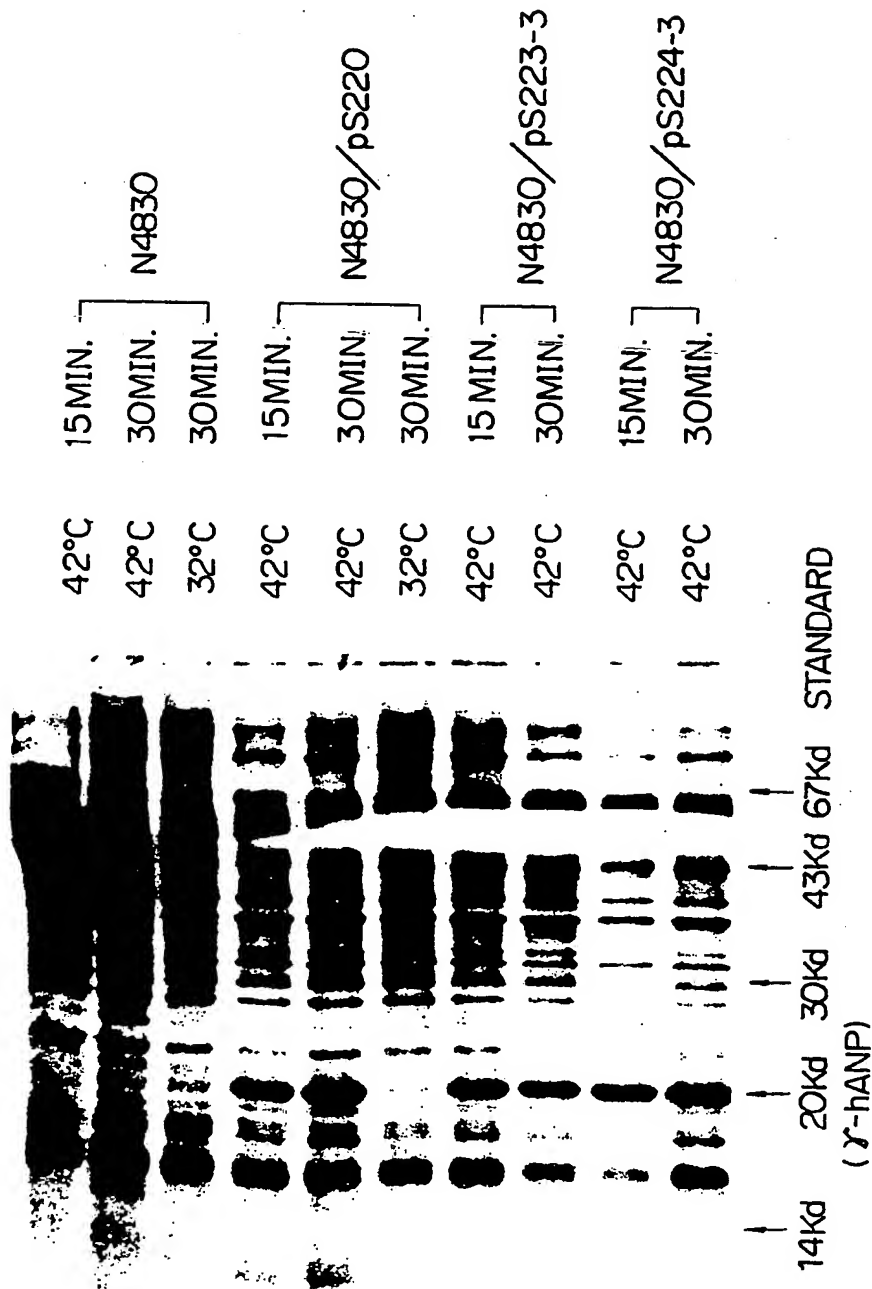


Fig. 8

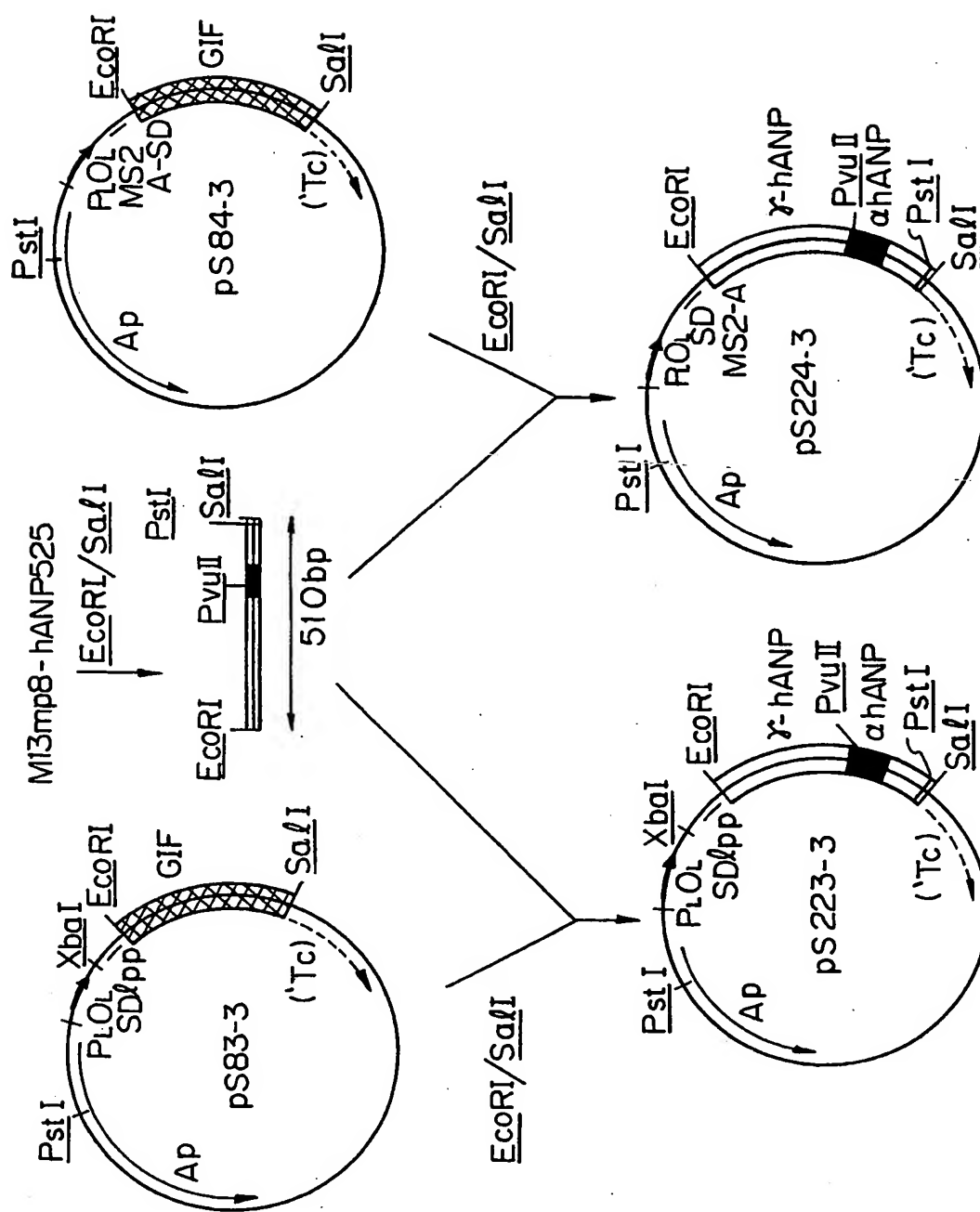
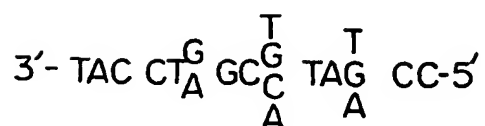
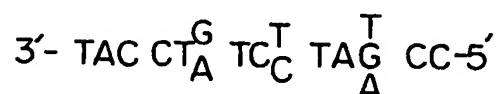


Fig. 9

Met Asp Arg Ile Gly



PROBE I



PROBE II

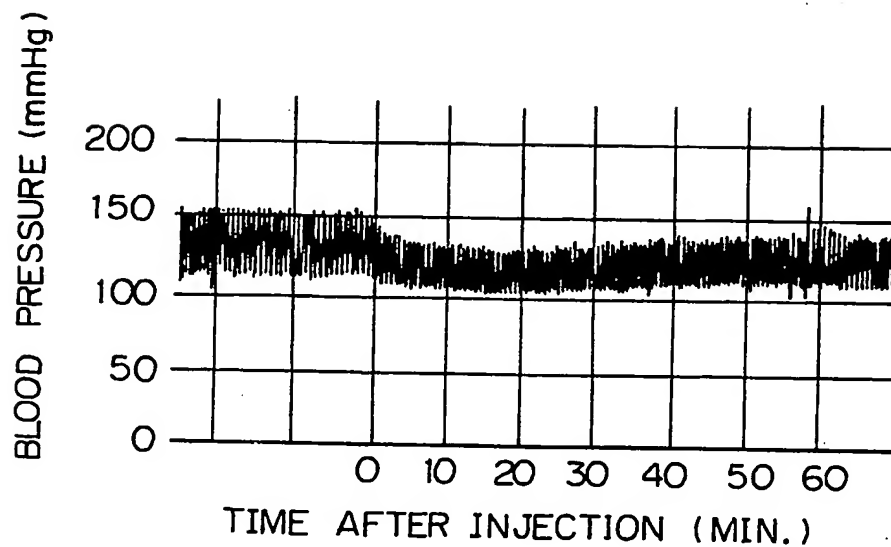
Fig. 12

Fig. 10

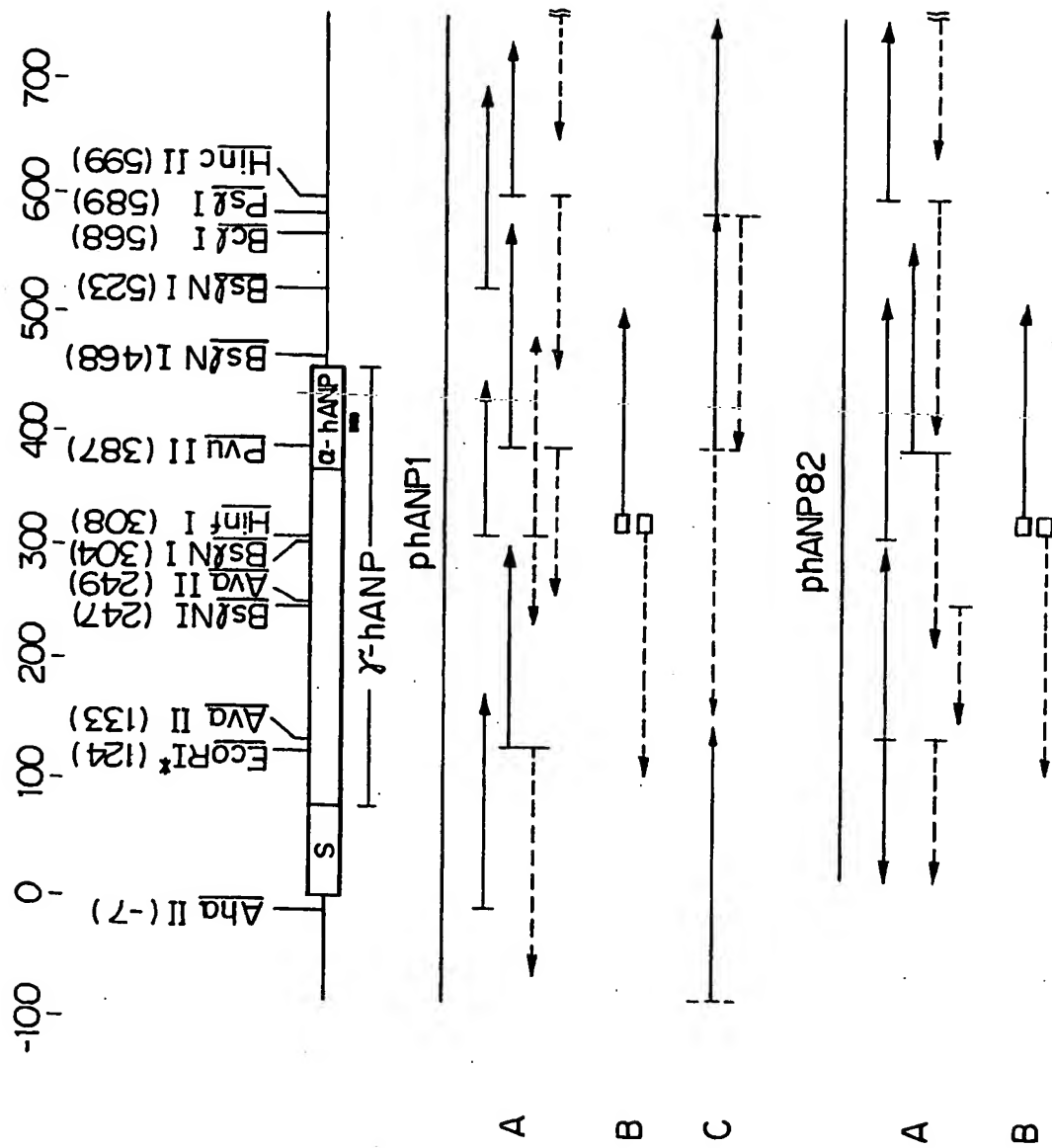


Fig. 11

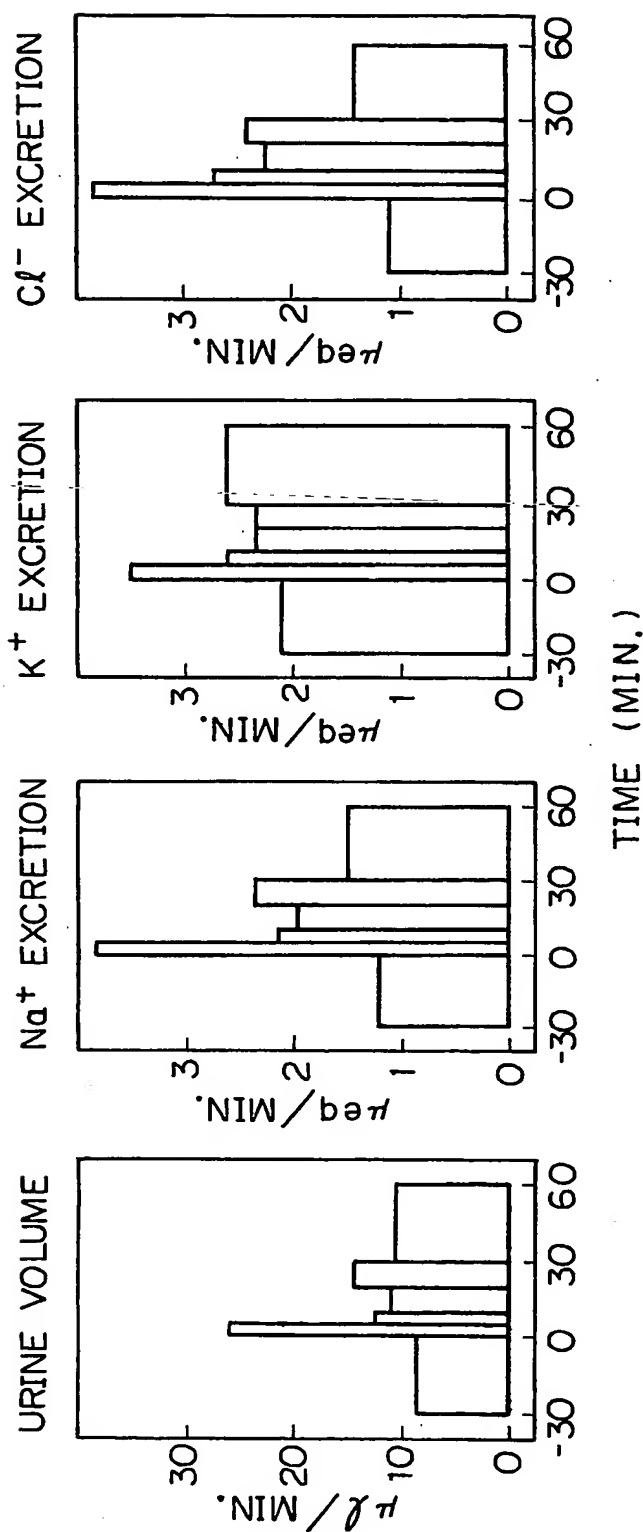


Fig. 13

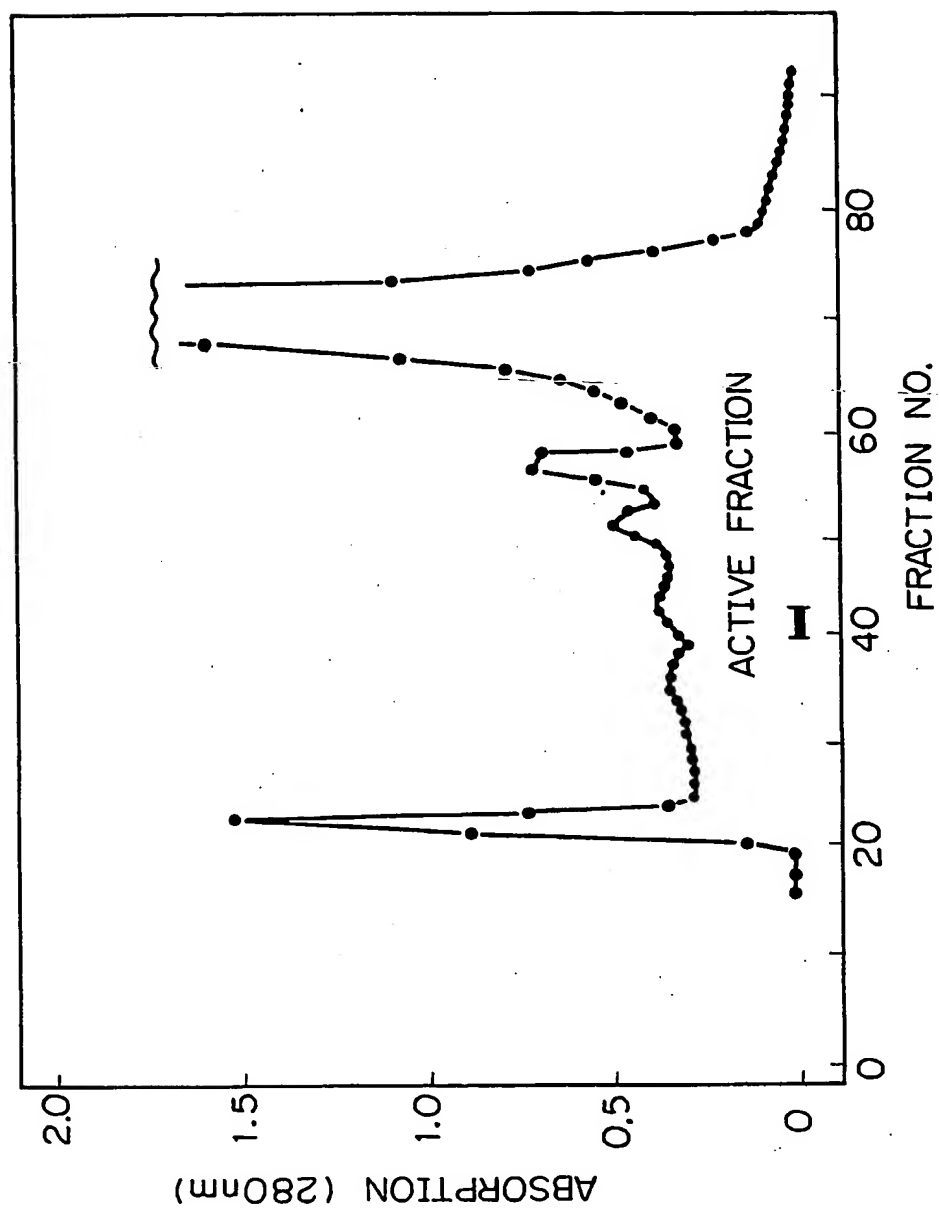


Fig. 14

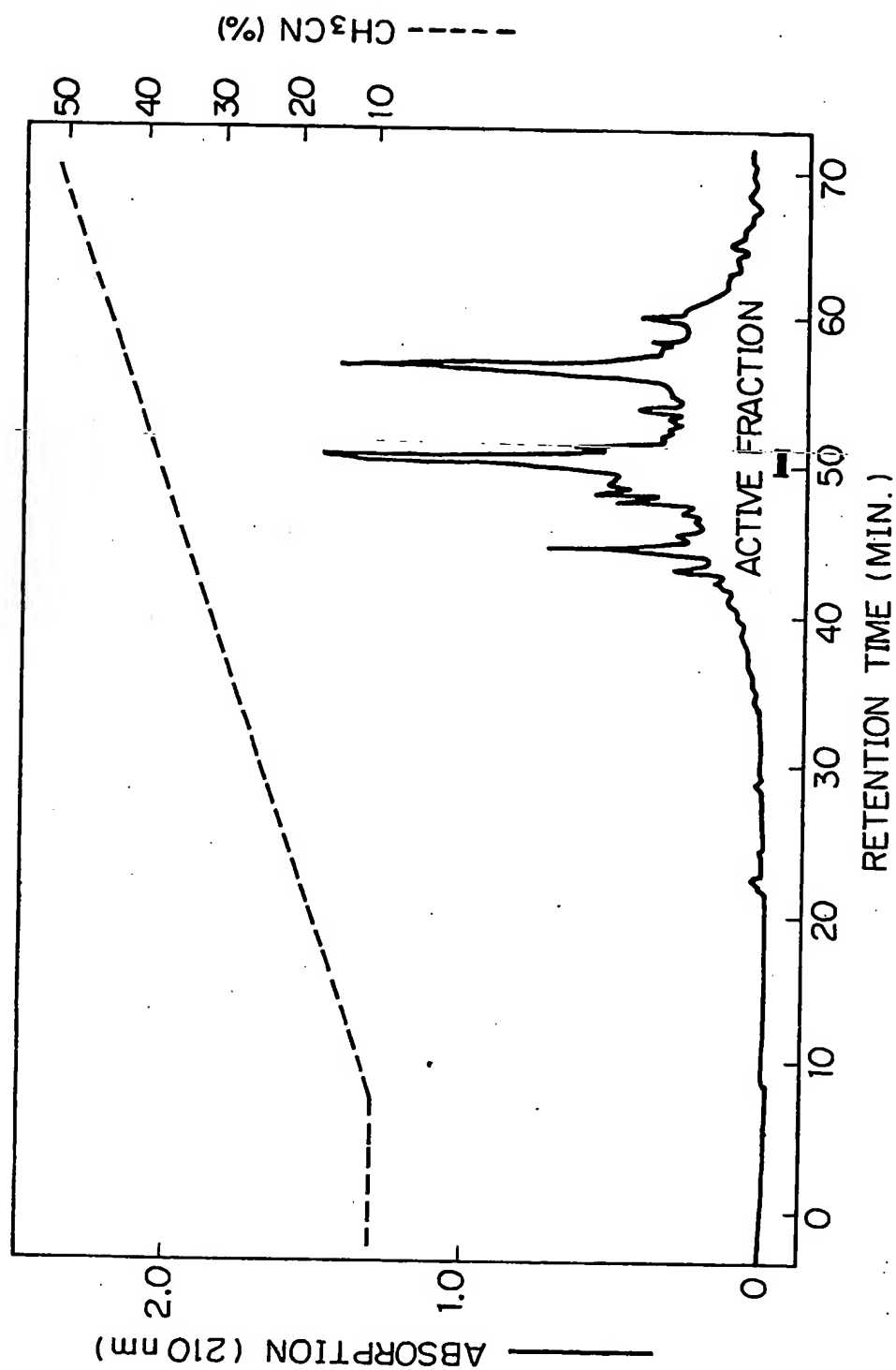


Fig. 15